EVALUATION OF HEAVY METAL POLLUTION AND ITS ECOLOGICAL RISK IN WATER FROM THE SASOLBURG REGION

BY

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DEDICATION

My study is devoted to a lot of people who are part of my life and who helped me be the person I am today. I am glad that they have witnessed me complete my MSc.

- Firstly, I would love to dedicate my research to my parents Sekonyela Andries Mollo and Nomahlubi Mollo for their love, support, strength, and never-die attitude of reaching for your dreams. I would love to thank them for their prayers and always being there whenever ever they are needed.

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ABSTRACT

Pollution of heavy metals is a worldwide problem, and the evaluation of heavy metals and their exposure to the environment has attracted international attention due to the health risk towards plants, humans, and animals. The accumulation of these metals within the environment has increased due to anthropogenic activities such as fertilizers and pesticides, mining, and industrial development, among others. Thus, it is crucial that research should be conducted on the metals to understand their mobility, bioavailability, and toxicity in sediments and water. Therefore, the purpose of the study was to determine the concentration levels of trace/heavy metals in sediments and surface waters (for domestic and farming use) from Sasolburg (Free State, South Africa). Inductively coupled plasma – optical emission spectrometry (ICP – OES) was used to quantify the concentrations of Al, As, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V. For the bioavailability and determination of trace/heavy metals in sediments single extraction technique and pseudo-total digestion was used. To help evaluate the potential health risk of trace and heavy metals in sediments and water, environmental assessments were utilized such as enrichment factor (Ef), contamination factor (Cf), geo-accumulation index (Igeo), and pollution load index (PLI) to name a few.

Firstly, heavy metal accumulation was determined in surface water samples collected in the dry and wet seasons of 2019 and 2020 in Sasolburg (Meulsteenpan Lake, Coalplex stream, and Natref stream). Permissible guideline values from the South African Department of Water Affairs and Forestry (DWAF), World Health Organisation (WHO), and Environmental Protection Agency (EPA) were used to compare trace metal concentrations against guideline values for water quality. Selected physiochemical parameters were investigated (pH, electrical conductivity (EC), and total dissolved solids (TDS)) in these waters. ICP-OES was used to determine heavy metal concentrations. Results obtained from ICP-OES showed that the mean concentration of Fe and Al in the wet season in Meulsteenpan Lake was extremely above the permissible guideline values set by WHO and DWAF (domestic use) and for Mn, the mean concentration exceeded the permissible guideline values set by DWAF and EPA (domestic use) and DWAF (agricultural use). In Coalplex stream Mo exceeded permissible guideline values set by DWAF (agricultural use) for both dry and wet season, while Fe and Mn had a mean concentration above permissible guideline values of DWAF and EPA (domestic use) with

Mn exceeding guideline values set by DWAF (agricultural use) in the wet season. In Natref stream for the dry season As had a mean concentration above guideline values set by DWAF, WHO, and EPA (domestic use) and in the wet season Fe mean concentrations exceeded guideline values set by DWAF (domestic use), while Mn had a mean concentration above guideline values set by DWAF and EPA (domestic use) and DWAF (agricultural use). Environmental assessments were used to help determine or classify the amount of pollution in this study such as m-HPI, HEI, Cf, and m-C_d and results obtained showed that the quality of the water is contaminated by heavy metals.

Lastly, the bioavailability and mobility of trace and heavy metals were determined using an ultrasonic-assisted single extraction method in sediments. Four extraction solvents were used in this study (EDTA, $CaCl_2$, deionised water, and acetic acid). Results obtained showed that EDTA, deionised water, and acetic acid were able to extract most trace and heavy metals (of Al, As, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V) with the highest concentration from all elements. The pH of sediment samples was slightly acidic for Meulsteenpan Lake (6.34 and 6.47), Coalplex stream (5.42 and 5.43), and Natref stream (5.83 and 5.63) for both dry and wet season respectively. Environmental assessments were used to help evaluate environmental contamination such as PLI, Cf, m-Cd, Ef, and Igeo. Results obtained from environmental indices showed that sediment samples were contaminated with trace and heavy metals from anthropogenic activities and were bioavailable for human and plant intake.

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$CaCl_2$ – Calcium Chloride

CEC - Cation-Exchange Capacity

Cf – Contamination factor

DNA – Deoxyribonucleic Acid

DWAF - South African Department of Water Affairs and Forestry

EC – Electrical Conductivity

EDTA – Ethylenediaminetetraacetic acid

Ef – Enrichment factor

EPA - Environmental Protection Agency

HEI - Heavy metal evaluation index

HCl – Hydrochloric acid

HNO_3 – Nitric acid

iCAP – Inductively coupled Argon Plasma

ICP – OES = Inductively Coupled Plasma – Optical Emission Spectrometry

Igeo - Geo-accumulation index

m-C_d - Modified degree of contamination

m-HPI – Modified Heavy metal pollution

MAC - Maximum Allowable Concentration

PLI – Pollution Load Index

RNA - Ribonucleic Acid

TDS – Total dissolved solids

UK – United Kingdom

USA – United States of America

USEPA – United States Environmental Protection Agency

WHO - World Health Organization

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

Poor water quality has a massive impact on human health, particularly those carrying bacteria, vectors such as diarrhea, and toxic elements (Genthe *et al.*, 2018). The World Health Organization (WHO 1998) report emphasized the impact of water on indigenous people, and that eight hundred million out of one billion people who do not use healthy water are living in poorly developed communities (USEPA, 2004). The involvement of surface water in these areas is essential since people depend on it for domestic use, such as laundry, cooking, drinking, and agricultural use (Odukoya *et al.*, 2017). Through the growth of industries, a vast volume of wastewater containing harmful pollutants such as heavy metals are periodically deposited and released into waterways (Peng *et al.*, 2015). "Heavy metals" are metals that are relatively dense with high atomic weights from 63.5 to 200.6 and a density greater than 5 g/cm^3. These metals are unfriendly to the environment and its surroundings. The term heavy metals are associated with metals such as aluminum, arsenic, lead, zinc, mercury, cadmium, cobalt, and iron to list a few, and are regarded a public concern by World Health Organization (WHO) (Chavan *et al*, 2016*;* Abdullah *et al.*, 2019).

The largest contributions of heavy metals to the environment is the mining sector and other industries. Apart from anthropogenic sources, heavy metals are introduced naturally through forest fires, weathering rocks, erosion, volcanic activities, and surface winds which through their interactions with the environment carry and redistribute heavy metals (Kobielska *et al.*, 2018; Shawai *et al.*, 2017; Akinwekomi *et al.*, 2016). Furthermore, soil is another known source of heavy metal introduction to surface waters by rain, which affects fish and birds that contribute to the food chain (Owa, 2013). Heavy metals from anthropogenic activities have severe effects on surface water systems and soil and are introduced primarily via three routes to the environment. That is the removal of atmospheric particulates, the release of sewage sludge and sewage waste, automotive pollution, mineral mining, oil, and gas production and the combustion of fossil fuels. (Shawai *et al.*, 2017 ; Oyewo *et al.*, 2020).

The existence of heavy metals in the mining sector is inexorable due to the saturation of their ores throughout the globe, and particularly in South Africa. From the waste created by

burning fossil fuels the most common heavy metals found from areas located next to mines are beryllium, cobalt, mercury, molybdenum, nickel, antimony, selenium, tin, vanadium, arsenic, chromium, copper, manganese, and zinc (Shawai *et al.*, 2017; Oyewo *et al.*, 2020). As such, the growth of industries increases the growth of these toxic metals to the environment, therefore it increases their mobility and bioavailability in the environment (Shawai *et al.*, 2017; Oyewo *et al.*, 2020). The bioavailability of these metals has several effects on surface waters and soil, such as the ability to change their concentration and physical properties (temperature, phase association, adsorption, and sequestration) (Feng *et al.*, 2019; Yedjou *et al.*, 2012).

These pollutants could be toxic to humans, marine organisms, and the environment, and long-term exposure to these metal ions may trigger elevated rates of gathered toxic substances in the body, which may cause disorders and eventually fatality (Zhong *et al.*, 2018; Peng *et al.*, 2015). For the human body, some of these heavy metals are essential for metabolic activities like chromium (Cr (III)), zinc (Zn), copper (Cu), selenium (Se), iron (Fe), manganese (Mn), cobalt (Co), and molybdenum (Mo). While other metals such as lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), antimony (Sb), and thallium (Tl) are non-essential to the human biological system (Odukoya *et al.*, 2017). Unpleasant health effects and changes to the biological system of humans and animals are associated with each metal and its chemical composition (Milenkovic *et al.*, 2019). Cellular organelles and components such as cell membranes, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and certain enzymes involved in metabolism have been known to be affected by the presence of heavy metals in the biological system. These metal ions interfere with cellular constituents like DNA and nuclear proteins, causing DNA damage and conformational changes that can lead to modulation of the cell cycle or carcinogenesis (Yedjou *et al.*, 2012).

1.1.1 Cadmium

Cadmium is a naturally occurring element with an oxidation state of +2 and has similar chemical properties to zinc (WHO, 2011). Commercially, cadmium is used extensively in batteries, pigments, coating, and alloy industries (Sharma *et al.*, 2015). Cadmium is known to be toxic/harmful to fish, humans, and various aquatic organisms (Fatoki & Awofolu, 2003). It has a life span of 10-35 years and is found in the kidneys of humans (WHO, 2017). It has been verified that cadmium has the potential to cause cancer through inhalation and is regarded as a harmful

element to humans by IARC (WHO, 2017). Some health effects of Cd include mutagenic, carcinogenic, and teratogenic (Fatoki & Awofolu, 2003).

1.1.2 Aluminium

Aluminium is natural and one of the most sumptuous components found on the Earth's crust (DWAF, 1996). There is no known important role for aluminium and it is not important for growth or reproduction (Inan-Eroglu & Ayaz, 2018). In water treatment methods, coagulants such as aluminium salts are used to reduce organic matter, colour turbidity, and microorganism concentrations (WHO, 2017). Exposure to Al primarily arises from food, environment, and occupational exposure (Inan-Eroglu & Ayaz, 2018). Excessive aluminium intake may lead to adverse health effects and has some negative effects on some organs of the body such as the hemopoietic system, the nervous system, and the bones. Furthermore, aluminium is regarded as carcinogenic and it is proposed that it has an impact on the occurrence of Alzheimer's dementia (Stahl *et al.*, 2017).

1.1.3 Chromium

Chromium is a naturally existing element made from three stable isotopes (^{52}Cr, ^{53}Cr, and ^{54}Cr), with an atomic mass of 24 in the periodic table. Chromium has multiple oxidation states from 0 to VI, (Lunk, 2015; Shekhawat *et al.*, 2015; Rakhunde *et al.*, 2012). Industrially chromium is used in metallurgical applications, manufacturing of stainless steel, and in the production of chromium chemicals (Barnhart & pharmacology, 1997). In nature, chromium is found in two oxidation states Cr (III) and Cr (VI). Cr (III) is the most stable and is found in the metabolism system of humans. Cr (III) is an essential element for the human biological system it merges with other enzymes in transforming sugar, proteins, and fat (Rakhunde *et al.*, 2012). Cr (VI) is the second most stable oxidation state from chromium and can combine with oxygen to make chromate or dichromate (Shekhawat *et al.*, 2015). IARC has considered Cr (VI) as carcinogenic to humans. Exposure to Cr (VI) has several health effects as it can cause skin rash, nose irritations, weaken the immune system, and nose bleeds (Rakhunde *et al.*, 2012).

1.1.4 Copper

Copper is a crucial trace element that has principal roles in several physiological purposes in the nervous, hematological, cardiovascular, reproduction, immune systems (Hefnawy & El-Khaiat, 2015), and it is also important for infant development, and growth (Stern & Environmental Health, 2010). Copper is one of the most widely used and naturally occurring metals in the Earth's crust. Most rocks and minerals in the Earth's crust consist of the metallic element and are located as pollutants in mineral ores. Copper toxicity is based on circumstances of municipal water purity. Its toxicity increases when there is a reduction in the amount of dissolved calcium, magnesium, and oxygen in the water (DWAF, 1996). Exposure to extremely high levels of copper has harmful effects on the liver, kidney, anemia, immunotoxicity, and developmental toxicity (Dorsey & Ingerman, 2004). The liver is the main organ that is targeted by excessive copper levels (Stern & Environmental Health, 2010). Damaged liver effects have been noted in patients who have genetic disorders like Wilson's disease, Indian childhood cirrhosis, and idiopathic copper toxicosis. These are hereditary disorders, which cause copper to collect in the liver and the latter two syndromes are related to prolonged exposure to copper (Dorsey & Ingerman, 2004).

1.1.5 Manganese

Manganese is naturally occurring in surface waters, groundwater, mainly in free oxygen systems (WHO, 2003) aka cotruvo, and an abundant amount is present in suspended and absorbed forms (DWAF, 1996). It exists as salt and mineral in aquatic ecosystems and is usually associated with iron compounds (WHO, 2017). Water resources with large amounts of manganese have an undesirable taste and stain sanitary goods and laundry at levels exceeding 0.1 mg/L (WHO, 2017). In the human body, manganese is responsible for some metabolic functions such as human development, activation of certain enzymes, immunological system function, and reproductive hormone function (Santamaria, 2008). There exist minimal reports on manganese toxicity from water and food sources (Aschner *et al.*, 2002). Many studies are concerned with the inhalation of manganese because of the vast number of employees who are exposed to it and prolonged exposure to manganese toxicity via inhalation may have harmful effects on the lungs

and may accumulate in the brain and this may lead to brain disease which may be comparable to Parkinson's disease (Röllin & Nogueira, 2019).

1.1.6 Molybdenum

Molybdenum is a vital trace element for many organisms including humans, animals, and plants. One of molybdenum's principal roles is to act as a co-factor for several enzymes that cause chemical reactions involved in the transportation of nitrogen, carbon, and sulfur (Smedley & Kinniburgh, 2017). Molybdenum is a metal that is mostly found in soil and is employed in the production of special steels such as the manufacturing of tungsten and pigments (WHO, 2017). In drinking water, concentrations of molybdenum must not exceed 10 ug/L. However, in previous studies, it has been noted that levels of up to 200 ug/L have been found in surface waters located next to mining facilities (WHO, 2003). Uncontrollable large amounts of molybdenum intake are harmful to the human body, even though minimal evidence in symptoms exists (Smedley & Kinniburgh, 2017). Novotny & Medicine, (2011) revealed from a study conducted in Armenia that high intakes of molybdenum through food have been reported and an estimated intake of about 10 mg/day to 15 mg/day. High concentrations of serum uric acid and high levels of xanthine oxidase tissue were observed from the community, and the health effects of molybdenum were painful joints, gout-like symptoms, and hyperuricosuria (Novotny & Medicine, 2011).

1.1.7 Iron

Iron is an abundant element in soils and rocks, mainly in insoluble forms (USEPA, 2001). As rainwater enters the soil, it is enriched with iron contents that will be dissolved and carried out into groundwater. Anaerobic groundwater contains soluble ferrous iron (Fe^{2+}) that is carried off to water reservoirs and other drinking water sources (Mandour, 2012). In humans, iron primarily exists in diverse forms connected to proteins such as heme compounds (hemoglobin or myoglobin), heme enzymes, or nonheme compounds. It is responsible for the transportation of oxygen around the human body by using hemoglobin and myoglobin as well as to produce heme enzymes and other iron existing enzymes involved in electron transfer and oxidation reductions (Abbaspour *et al.*, 2014). Excessive iron ingestion can contribute to hemochromatosis, with the accumulation of iron having negative impacts on muscle tissues. Poisoning from drinking water

or surface water is unlikely since abundant concentration levels of iron do not exist (DWAF, 1996).

1.1.8 Arsenic

Arsenic is semi-metallic and is typically found in the earth's crust (Singh *et al.*, 2007). Arsenic is common to sulfides, metal arsenides, arsenates in several oxidation states; namely III, IV, V, and -III. The most common forms of arsenic primarily are arsenic (III) and arsenic (V) and occur in surface waters. Concentrations of arsenic species are dependent on the alkalinity and oxidation/reduction potential of the water (DWAF, 1996). Chemical compounds that contain arsenic are used commercially to produce transistors, lasers and semiconductors, glass, pigments, textiles, paper, metal adhesives, and ammunition. Arsenic is typically in the range of 1 to 2 ug/L in groundwater. However, in areas of volcanic rock, sulfide mineral deposits, and mining areas concentrations may be high (WHO, 2003). Arsenic is not a crucial element in humans (Council, 1999). However, it is regarded toxic and according to previous reports, arsenite (As (III)), is more toxic than arsenate (As (V)) (WHO, 2017). Arsenic is very harmful to humans even at very low concentrations, and inorganic arsenic is registered as a human carcinogen (USEPA, 2001).

1.1.9 Nickel

Nickel is a smooth, pure, and ferromagnetic metal. It takes up five isotopic forms naturally: 58 (67.8%), 60 (26.2%), 61 (1.2%), 62 (3.7%), and 64 (1.2%) (WHO, 2005). It is a naturally occurring element and is omnipresent in the environment. It is found in soil, water, air, plants, and animals in different forms of nickel compounds (Stannard *et al.*, 2017). Food is a leading source of nickel intake for people who are not exposed to the metal via work-related issues or non-smokers, while water is a minimal contributor (WHO, 2017). Nickel enters the human body mainly through inhalation and ingestion owing to work exposures and lifelong diets, large amounts of nickel may be stored in the human body in various ways (Denkhaus & Salnikow, 2002). Nickel is not known to be a crucial element in humans, and it is not fully understood how nickel compounds are metabolized by the human body. However, it is detrimental to human health if excessive exposures to nickel compounds occur. While nickel accumulation can lead to lung fibrosis, cardiovascular disease, and kidney disease in the body through chronic exposure, serious concerns relate to nickel carcinogens (Denkhaus & Salnikow, 2002).

1.1.10 Lead

Lead is one of the most common heavy metals elements and accounts for 13 mg/kg of the earth's crust (WHO, 2011). It is listed by the USEPA as a cumulative hazardous poison to all living beings and exists in several oxidation states (0, II, IV, and I) that have an important contribution to the environment (DWAF, 1996). Metallic lead is used in commercial products like pipes, lead solder, radiation shields, electric storage batteries, and sheaths for electrical cables (Juberg, 1997). Prepared food has low yet significant concentrations of lead (WHO, 2011). However, when exposed to lead, the metal bio-accumulates in living tissue and has plenty of health effects associated with it, these include impaired kidney function, high blood pressure, neurodevelopmental effects, mortality, and unfavorable results of pregnancy (WHO, 2017).

1.1.11 Antimony

Antimony is a silver-white metal (atomic number 51) and is a naturally occurring element found in the earth's crust (Sundar *et al.*, 2010). Heat and electricity are not easily conducted by antimony (Schulz *et al.*, 2018). Due to its inflexibility, the metal has limited specific applications but when merged with either copper, lead, or tin it forms very hard alloys (WHO, 2003). Since its discovery by the 14th-century alchemist of Rupescissa, antimony compounds have been utilized as medicines primarily for treating two parasitic diseases, leishmaniasis and schistosomiasis (Sundar *et al.*, 2010). Antimony concentrations usually vary between 0.1 and 0.2 ug/L in groundwater and surface water. Furthermore, extremely high concentrations of antimony rarely exist except for those that are located next to mines (WHO, 2003). Excessive exposure to the metal is associated with symptoms like headache, colic, constipation, distaste for food, loss of appetite, dizziness, and weight loss (Cooper *et al.*, 2009).

1.1.12 Selenium

Selenium is a naturally occurring element found in the earth's crust, mostly in association with sulfur minerals. Average concentrations of selenium found in the earth's crust are between 50-90 ug/kg, although excessively higher amounts have been noted and are associated with volcanic, sedimentary, and carbonate rocks (WHO, 2011). In animals and humans, selenium is a required (essential) nutritional and health element that plays a crucial role in preventing liver disorders in

experimental animals attributed to the lack of vitamin E, sulfur amino acids, and selenium (Majeed & Badmaey, 2005). Concentration levels of selenium in surface water and groundwater are between 0.06 ug/L to 400 ug/L (WHO, 2011). However, higher concentrations of selenium may be found in waters that are subjected to industrial activities (WHO, 2017). Excessive exposure to selenium is harmful to the body and inhalation of selenium gas, selenium dioxide, or hydrogen selenide has detrimental effects such as irritation of the mucous membranes of the eyes and upper respiratory tract, headache, dizziness, dyspnea, vomiting, nausea, garlic odor of the breath, and fatigue (Fan & Kizer, 1990).

1.1.13 Cobalt

Cobalt is a hard-silver grey metal located in group 9 on the periodic table (atomic number 27). It is a naturally occurring metal and is the 33rd most abundant element found in different surroundings such as surface water, air, wastewater, groundwater, soil, and sediment (Kim *et al*, 2006; Nagpal, 2004). Cobalt is a very crucial metal in the manufacturing industry and has several uses such as in batteries and superalloys. In batteries, cobalt is used in the engineering of cathode materials, lithium-ion, nickel-cadmium, and nickel-metal-hydride batteries that are used in devices, electric cars, capacitors, and power tools (Schulz *et al*, 2018). Superalloys are high-temperature alloys that are created, where a relatively high mechanical stress is experienced and surface stability is needed frequently (Schulz *et al*, 2018). Cobalt which is an essential component of the B12 vitamin complex is an essential element for humans even at trace amounts. It is an important element in the growth of marine plants/species such as diatoms, cryophytes, and dinoflagellates. Although its importance is higher in humans, animals, and plants excessive concentrations of cobalt may be toxic and lead to death (Nagpal, 2004).

1.1.14 Strontium

The fifteenth most abundant metal in the earth's crust is strontium, which is mainly found in groundwater and some surface waters near coastal and mountainous regions (Chaalal *et al*, 2015). Strontium has four naturally occurring isotopes (^{84}Sr, ^{86}Sr, ^{88}Sr, and ^{87}Sr). In which, ^{87}Sr is the only isotope produced radiogenically from the decay of ^{87}Rb (Ladegaard-Pedersen *et al*, 2020). Strontium is an essential element in human bones and teeth. The amount of Sr presented in drinking water is not regularly monitored, therefore research has shown that excessive intake

or ingestion of Sr contributes to unnatural skeletal developments and bone calcification (Langley *et al*, 2009). Due to its potential health threat towards humans, in the year 2013, the USEPA stated that it requires a report of any drinking water with a concentration greater than 0.3 ug/L of Sr (Ladegaard-Pedersen *et al*, 2020). Strontium has several industrial applications such as the removal of lead from zinc electrolytic solutions, manufacturing of different goods such as glass, ceramic, permanent magnets, and aluminium alloys. Strontium salts are also used in cancer therapy or as natural health products mainly to assist with bone health (CA Committee on Drinking Water, 2018).

1.1.15 Thallium

In the periodic table, thallium lies between mercury and lead and is found in group 13 of the periodic table and has two stable isotopes and they are ^{203}Tl and ^{205}Tl. (Belzile & Chen, 2017). William Crookes an English chemist was the first person that discovered thallium in 1861 (Xu *et al*, 2019). Thallium is found at very low trace concentrations in nature (generally < 1 ug/g) and is a non-essential element that is highly toxic towards humans compared to mercury, cadmium, lead, copper, and zinc (Belzile & Chen, 2017). Due to its high toxicity, the number of uses for thallium are limited. In the 1920s thallium was used as a rodent killer until it was prohibited in the USA in 1972 (Xu *et al*, 2019). In the electronics industry, thallium is used in photoelectric cells. Thallium is also used in the manufacturing of a unique glass that has a high index of refraction and a low-melting glass that becomes liquid at approximately 125 K (Belzile & Chen, 2017).

1.1.16 Vanadium

Vanadium is the 14th most abundant metal in nature and is silver in colour. It is found in-between titanium and chromium on the periodic table and has two stable isotopes (^{50}V and ^{51}V) with an abundance of 0.25 % and 99.75%, respectively (Pourret & Dia, 2018). Vanadium has five valence electrons, with maximum oxidation of +5, with a series of oxidations states from 0, +2, +3, +4, and +5, with V(IV) and V(V) being the most stable (Meisch & Bieling, 1980). Vanadium is a crucial element and is largely distributed in the earth's crust. For green plants such as algae,

vanadium has a positive impact on the increase of chlorophyll and oxygen evolution during photosynthesis (Chasteen, 1983; Meisch & Bieling, 1980). In trace amounts vanadium is an essential element for cell growth, but when excessive amounts are indigested it may cause adverse effects since its toxicity towards humans has harmful effects (Pourret & Dia, 2018; Meisch & Bieling, 1980).

1.2 PROBLEM STATEMENT

A global concern affecting human and animal health is water quality. A significant challenge for the survival of life is inorganic and organic chemical pollution in surface water and groundwater (Saeed & Shaker, 2008). Inorganic contaminations are typically mineral elements, such as metals, salts, and minerals. Research has pointed out that inorganic contaminants have been introduced into the ecosystem in large quantities due to anthropogenic activities (Masindi & Muedi, 2018). Heavy metals are a typical example of inorganic contaminants and different pathways of heavy metal pollution are illustrated in Figure. 1.

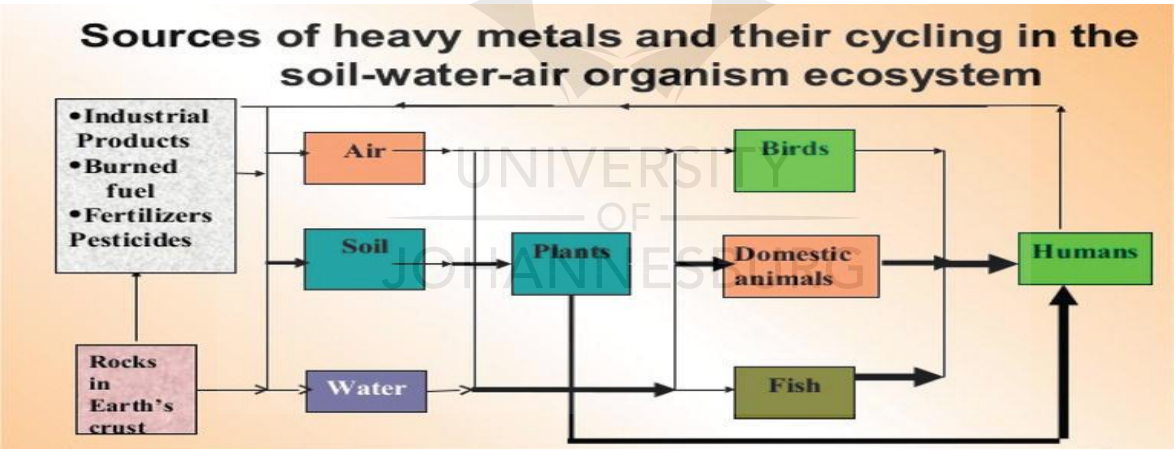

Figure 1.1: Schematic diagram of the mechanism of heavy metals in air, soil, water, plants animals, and humans.

The introduction of these heavy metals into our ecosystem is accomplished by a variety of natural mechanisms, such as soil erosion, volcanic eruptions, and microorganism activities, and through anthropogenic activities which include mining, wastewater, and industrial contamination (Florea *et al.*, 2004). Such metals are non-biodegradable and accumulate in the human body

through various methods causing unfavourable health issues. This in turn may impair mental health, affect the cardiovascular system, liver, kidney, and other life-threatening organs. Furthermore, prolonged exposure to heavy metals may lead to muscle, physical, and neurological degenerative processes, which may imitate other diseases like Parkinson's and Alzheimer's diseases (Jaishankar *et al.*, 2014). Also, extended exposure or interaction with such heavy metals and their compounds can cause damage to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), cause mutation, impersonate hormones thereby disrupting the endocrine system, genitalia, ultimately leading to cancer (Järup, 2003).

1.3 HYPOTHESIS

The introduction of heavy metals into environmental surroundings (water and sediments) lowers its quality. Waste carrying heavy metals from anthropogenic activities has adverse effects on environmental quality from areas located next to industrial sectors.

1.4 AIM AND OBJECTIVES

1.4.1 Aim

This study aimed at assessing the concentration levels of heavy metal contamination in selected streams around Sasolburg, Free State (South Africa). Surface water is often used without prior treatment by residents in the selected study area for domestic, agricultural, and recreational activities. Contamination, distribution, and health effects were looked at in water and soil using various pollution assessments to evaluate the contamination quality in the study area. The main trace and heavy metals that were studied for this area were Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V and quantified using the inductively coupled plasma – optical emission spectrometry (ICP-OES).

1.4.2 Specific Objectives:

- Quantification of the concentration levels measured from the water samples collected from Sasolburg, Free State (South Africa) and to evaluate the environmental impact caused by these metals by using various pollution indices.

- Assess heavy metal concentration in sediment samples collected from Sasolburg, Free State (South Africa) by using ultrasound-assisted single extraction and pseudo-total digestion techniques aided by the ICP-OES. The use of different pollution assessments to help evaluate the mobility and bioavailability of these trace metals.

1.5 THESIS/DISSERTATION OUTLINE

• **Chapter 1** focuses on the introduction or background of heavy metals, together with problems and justification of the study. The motivation for this study, hypothesis, and objectives.

• **Chapter 2** provides a detailed literature review on methods for sample preparation and using single extraction technique. The chapter also gives a detailed highlight on pollution and contamination indices used for water and soil samples.

• **Chapter 3** gives detailed highlights on the evaluation of surface water collected from Sasolburg, Free State (South Africa). Comparing the concentration levels analysed with those recommended by EPA, DWAF, and WHO. Use various pollution indices to help evaluate the water quality.

• **Chapter 4** looks at the use of ultrasound-assisted single extraction and pseudo-total digestion techniques in soil samples, with different solvents used as extractants. Assess the environmental impact and quality using indices

• **Chapter 5** presents a summary of Chapters 3 & 4 the major findings of this work and the conclusions drawn from the results obtained as well as recommendations and future work.

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CHAPTER TWO:
LITERATURE REVIEW

2.1 HEAVY METAL CONTAMINATION AND ITS EFFECTS ON SOIL, AQUATIC ECOSYSTEMS, AND PLANTS

Due to the rapid growth rate of the industrial revolution, heavy metal pollution in the environment has become a major concern. This has caused environmental quality to depreciate while having adverse effects on the ecosystem (Naggar *et al,* 2018; Rai, 2018). In nature, the most affected habitats are aquatic ecosystems that are used as waste disposable systems by industrial and urban sectors (Naggar *et al,* 2018). This influences the soil biota from these areas, resulting in a decrease in vegetation and diversity in soil microbial (Ashraf & Ali, 2007). Among these metals, lead (Pb) is one of the majors contaminates causing soil pollution. A study conducted by Bhattacharyya *et al,* (2008) reported that high concentrations of lead in soil reduce soil productivity, while lead deficient soil has negative effects on many plant processes such as photosynthesis, mitosis, and water absorption with physical effects such as short brown leaves, dark green leaves, stunted foliage, and brown short roots. The absorption of these metals by plants and soil poses a potential risk to human health (Khan *et al,* 2008). In relation to the above-mentioned concerns, this chapter reviews the effects of heavy metal pollution in soil, aquatic ecosystems, and plants.

2.1.1 Effects of heavy metal on soil

The most significant problem in the world is the contamination of soil by heavy metals. Contamination of soil by heavy metals leads to detrimental effects that could impact essential microbial processes thus reducing the amount and activity of soil microorganisms. Furthermore, the non-biodegradability properties of heavy metals in soils have a significant effect on vegetation, animals, and human beings. Generally, the pH of the soil is used as a chemical indicator to investigate its quality. Studies have reported that in acidic soils exposure to heavy metals results in serious health effects and consequently impacts other characteristics of soil (Jiang *et al,* 2012; Fan *et al,* 2014; Hinojosa *et al,* 2004). For instance, chromium (III), copper (II), cadmium (II), and lead (II) are known to have strong hydrolysing capacities and this, in turn, can dramatically reduce the pH of the soil (Jiang *et al,* 2012; Fan *et al,* 2014; Hinojosa *et al,* 2004; Yu *et al,* 2006; Sankhla *et al,* 2016; de Mora *et al,* 2005). He *et al, (*2015) reported that there are several biogeochemical factors either than pH that are

considered as contamination indicators such as biochemical indicators, microbial indicators, and soil animal indicators (earthworms). Franzaring *et al,* (2010) noted that plants like the Beula pendula tree, Fraxinus excelsior tree, and Malus Domestica tree serve as chemical indicators for lead (Pb) contamination, with increased contamination, malfunction of leaves, and necrosis is experienced. Since most heavy metals are non-biodegradable, they easily become contaminants in the soil, because the number of heavy metals released from anthropogenic systems is far greater compared to natural metals. They translocate from mining facilities to public environmental locations with greater chances of direct contact. The amount of waste released from industrial sectors containing trace/heavy metals has higher concentrations of these elements while those found in nature are lower (D'amore *et al,* 2005).

During outdoor activities, children under the age of three, are likely to ingest soil. They have been considered vulnerable to pollution especially from soil contaminated with heavy metals, with the intake of lead (Pb) being absorbed five times faster through their gastrointestinal system. In some countries, adults tend to consume soil due to cultural reasons. When consumed, some contaminates are dissolve in the lining of the mouth as they are ingested, whereas others are swallowed and transported to the gastrointestinal system (Science Communication Unit, 2013). Agricultural employees are likely to inhale soil while working, this is caused by small soil particles that are released and are easily inhaled. Once these particles carrying contaminates enter through the lungs there is a likelihood of them being absorbed into the bloodstream. This is a far less critical exposure compared to ingestion but is highly significant to individuals exposed to it daily (Science Communication Unit, 2013).

2.1.2 Effects of heavy metals in aquatic ecosystems/environment

Heavy metals such as Pb, Cd, Hg, Sb, Tl, and As, among others are toxic even at trace levels and can remain within the environmental compartments for a long time after introduction (Peng *et al*, 2008: Woo *et al,* 2009). When heavy metals are introduced to aquatic ecosystems they normally attach to matter, which will eventually settle and be absorbed by sediments (Peng *et al*, 2008: Woo *et al,* 2009). Since a large part of trace metals within aquatic ecosystems are correlated with sediments in areas where requirements of water quality are not surpassed, environmental contamination by metals is possible while organisms in and around the sediments are adversely affected (Singh & Kalamdhad, 2011). This in turn has detrimental effects on the aquatic ecosystem by causing an ecological imbalance and

affecting aquatic organisms. In aquatic organisms, fish are the inhabitants of this environment who cannot escape these adverse effects. These metals then make it through the food chain by accumulating in aquatic organisms that are consumed by humans (Ay *et al*, 2009; Baby *et al* 2010). In a long run, due to their toxicity, the gradual and irreversible accumulation of these metals in different organs contributes to metal-related illnesses, thus endangering the aquatic biota and other organisms (Baby *et al*, 2010). In a study conducted by Li *et al*, (2018), it was noted that one of the primary sources of bone diseases in humans is the intake of heavy metals via the consumption of water and ingestion of aquatic organisms. In a study reported by Rai, (2008) in the Gangetic plain of Eastern Uttar Pradesh and the Western Bihar region of India, high concentrations of cadmium were recorded in wastewater, irrigation water, and crops causing carcinoma and gall stone to the community.

2.1.3 Effects of heavy metals in plants

For plant growth and development, certain heavy metals are required for this process, which enables them to accumulate essential (Co, Cu, Fe, Mn, Mo, Ni, and Zn) and non-essential (As, Cd, Hg, Pb, and Se) metals, while extreme levels may have adverse effects on plants (Djingova & Kuleff, 2000; Singh & Kalamdhad, 2011). When ideal heavy metal concentrations are exceeded, severe effects such as the destruction of the cellular structure due to oxidative pressure and inhibition of cytoplasmic enzymes are faced by plants (Djingova & Kuleff, 2000; Singh & Kalamdhad, 2011). In addition, plant growth is also affected by the negative impacts of extreme heavy metal concentrations. For example, if a decrease in soil nutrients is experienced, biological catalysts such as enzymes are affected. Such toxic effects contribute to a decrease in plant growth that may also lead to plant death (Schaller & Diez, 1991). Factors such as pH, temperature, moisture, organic matter, and the availability of nutrients are influential towards the accumulation and absorption of heavy metals in plant tissue (Singh & Kalamdhad, 2011). In a study conducted by Sharma *et al*, 2007 it was noticed that in summer the absorption and accumulation of Cd, Zn, Cr, and Mn in Beta vulgaris (Spinach) was greater, while in winter Cu, Ni, and Pb increased. A study conducted on the effects of mercury on some growth parameters of rice (*Oryza sativa* L) by Kibra, (2008) revealed that soil contaminated with mercury (1 mg/kg) had several adverse effects on rice plants such as the reduction in height, tiller, and panicle formation.

2.1.4 Environmental monitoring in South Africa

Environmental contamination from industries and other human activities in South Africa has been a growing concern and this has gained interest from numerous researchers across the country. A study in Mhlathuze Estuary, northern Kwazulu-Natal, South Africa was conducted by Izegaegbe *et al*, (2020) on metal accumulation in sediments. Metal concentrations from the estuary were generally low as measured against sediment quality guidelines, with only Ni and Cr exhibiting toxicity towards aquatic life. In Thohoyandou, Limpopo province, South Africa at Maina *et al*, (2017) performed a study on trace/heavy metal accumulation (Cd, Co, Cr, Cu, Mn, and Ni) on House Sparrows using them as bio-indicators due to their abundance and close interaction with humans. It was found that concentrations of cadmium were 8-18 times higher than the threshold limit posing health threat issues towards the House Sparrow and possibly other organisms. Edokpayi *et al*, (2016) conducted a study in Mvudi River, South Africa on the assessment of trace metal contamination (Al. Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in surface water and sediment. The concentration levels of Al, Cr, Fe, Mn, and Pb in the water samples were higher than the DWAF and WHO recommended levels for domestic water use. High concentrations of Al and Fe were also determined in sediment samples. Additional studies done on environmental monitoring in South Africa are listed in Table 2.1.

Table 2.1: List of studies performed on environment monitoring in South Africa

Title	Metals	References
Spatial and temporal variations in selected heavy metals in water and sediment from the Mhlathuze Estuary, Richards Bay. African Journal of Environmental Science and Technology	Al, Cr, Cu, Mn, Pb, and Zn	Mzimela *et al*, (2014)
Monitoring metals in South African harbours between 2008 and 2009, using resident mussels as indicator organisms	Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sr, U, and Zn	Wepener & Degger, (2020)
A spatiotemporal analysis of water quality characteristics in the Klip River catchment, South Africa	Al, As, Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, U, V, and Zn	Marara & Palamuleni, (2020)
Spatial assessment of heavy metals contamination in household garden soils in rural Limpopo Province, South Africa	As, Cd, Hg, Pbi	Kapwata *et al*, (2020)

Polysaccharides Based Adsorbent Used for the Assessment and Modeling of Metals Ions in Olifant's River Catchment, South Africa	Al, Ba, Ca, Cu, Fe, Mg, Mn, Na, Ni, Sr, Ti, and Zn	Atangana *et al*, (2020)
Metal bioaccumulation and energy biomarkers in tissues of two populations of Chiromantes eulimene from Richards Bay Harbour, South Africa	Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn	Majola *et al*, (2020)
The reach of human health risks associated with metals/metalloids in water and vegetables along a contaminated river catchment: South Africa and Mozambique	Al, As, Ba, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, Sr, U, V, and Zn	Genthe *et al*, (2018)

2.2 SOURCES OF HEAVY METAL POLLUTION

In most soils, plants, and living organisms trace metals are present and are defined as metals that exist in low concentrations (mg/kg or lesser) (Phipps, 1981). The introduction of trace metals into the environment is through natural and anthropogenic processes (Presser, 1994). Anthropogenic processes are man-made activities that contribute high concentrations of trace metals to the environment through industrial activities (mining) and the use of fertilizers (He *et al*., 2005).

2.2.1 Fertilizers:

In most agricultural ecosystems' fertilizers are used for the development and growth of crops, while most trace elements are found in fertilizers. Cadmium, Pb, and As are trace metals present in phosphorus (P) fertilizers such as triple superphosphates and calcium/magnesium phosphate, in which phosphate is an important nutrient for productivity and plant growth (Mortvedt & Beaton, 1995; Alkhader, 2015). Concentration levels greater than 50 mg/kg in fertilizers have been recorded and these metals are regarded as toxic and harmful (Mortvedt & Beaton, 1995; Alkhader, 2015). In previous studies conducted by Galadima, (2012) and Agwaramgbo *et al*, (2014), it was reported that in 2010 five hundred children were announced dead due to lead (Pb) poisoning, while over a thousand had serious health conditions. In literature, it has been revealed that some severe health problems such as kidney disease were caused by the exposure of As, Cd, and Pb in areas such as Sri Lanka (Jayasumana *et al*, 2015). In addition, it was noted that cadmium is extremely mobile and can accumulate in large quantities in plants without exhibiting signs of phytotoxicity. Cadmium is very toxic to human health and enters the food chain by accumulating in vegetables (Poder & Ramsey, 2005).

2.2.2 Mining

In South Africa's economical structure, mining is one of the largest contributors, with the country being popular for its resources such as manganese (Mn), chrome (Cr), platinum (Pt), vanadium (V), zirconium (Zr), and coal (Sedibe *et al*, 2017). The introduction of trace/heavy metals into the environment and its surroundings (soil, water, air, and plants) may be expected from mining sectors. Trace/heavy metals tend to absorb and accumulate in plants and food, which is ingested by humans and animals, posing danger to their biological health system (Zhuang *et al.*, 2009; Li *et al.*, 2014; Pan *et al.*, 2016; Navarro *et al.*, 2008; Xu *et al.*,

2013; Halim *et al*., 2015; Qu *et al.,* 2012; Goyer., 1996). The release of wastewater from mining sectors has detrimental effects on the environment, with these waters being used as irrigation water for agriculture (Liao, 2016). In a study by Requelme *et al*., (2003) conducted in Namibia (Ecuadorian Amazon) concentrations of mercury-added in the gold extraction process were between 1.7 and 2.7 mg/kg in soil and sediments. While in Tanzania and Zimbabwe, have experienced levels up to 5.35mg/kg of mercury in sediments next to gold mining facilities (Van Straanten., 2000).

2.3 CHEMICAL EXTRACTION METHODS OF METALS IN SOILS AND SEDIMENTS

For metal analysis in soils and sediments, chemical extraction methods are used to extract metals into an aqueous solution (Gupta & Aten, 1993). Soils and sediments are distinct matrixes, particularly in nature. Soil can be described as a three-dimensional thin layer that periodically reflects the effect of climate, plants, animals, organic matter, and rocks on the earth's crust (Cappuyns, 2012). Sediment is a matter that is carried by water and settles on the surface of the ground or water and can condense into rocks over time (Cappuyns, 2012). Trace elements exist in various chemical forms in soils and sediments which influence the mobility, toxicity, and bioavailability of these metals. Soil quality is assessed by evaluating the mobility, toxicity, and bioavailability of trace elements (Sihlahla *et al,* 2020). To provide valuable information about bioavailability and percentages of trace metal contamination present in soil and sediments, a single extraction method may be used. The single extraction method represents a quick, inexpensive, and convenient way of evaluating the mobility of trace elements in polluted soils and sediments (Sihlahla *et al,* 2020; Cappuyns, 2012). Single extraction method can be categorized into three groups: (i) Exchangeable (unbuffered salt solution); (ii) Organically complexed metal fraction (Chelation agents) (EDTA); (iii) and an acid-extractable fraction (acetic acid) (Bakircioglu *et al,* 2011).

In single extraction one of the most used extractants in unbuffered salt solutions (calcium chloride ($CaCl_2$)). Its prescribed dose for the method by countries around the world such as the Netherlands is 0.01 mol/L $CaCl_2$ (Zhang & Zhou, 2020). These extractants consist of poorly absorbed metals held on the solid surface due to inadequate electrostatic interactions, and metals that can be released by the ion exchange process (Bakircioglu *et al,* 2011). In soil-based chemistry, EDTA (strong chelating agent) is used extensively to assess the bioavailability of trace metals. The tolerance of EDTA towards complex metals is high

therefore major elements are also dissolved (Manouchehri *et al*, 2006; Cappuyns, 2012). In a study by Cappuyns, (2012), it has been demonstrated that EDTA removes amorphous iron (Fe) oxides, although this reaction has shown to be time-consuming in the presence of other metal-chelate complexes.

The most frequently used reagent in acid-extractable reactions is 0.11 mol/L acetic acid to assess metal concentrations in soil extraction techniques. pH is a key parameter of the extracts as it plays a crucial role in the selectivity of the reaction. The main role of 0.11 mol/L acetic acid is to free exchangeable elements and the dissolution of certain crystalline hydroxyl and carbonate-metals compounds (Miller *et al*, 1986; Tessier *et al*, 1979). In a study conducted by Sihlahla *et al*, (2020), the bioavailability and mobility of trace metals in agricultural soils were evaluated using extractants such as EDTA, $CaCl_2$, NH_4NO_3, deionized water, and CH_3COOH. Results obtained from single extraction demonstrated that Al, Fe, and Mn experienced higher extraction, with deionized water, acetic acid, and EDTA being the most effective extractants. In a study by Sahito *et al*, (2015) trace metals such as Cd, Cu, Pb, Fe, and Zn were assessed using a single extraction method to evaluate their mobility and bioavailability. Results obtained revealed that Cd had a higher concentration than all other metals. Table 2.2 lists some of the studies in which single extraction procedures have been applied for the investigation of bioavailable and mobile heavy metals.

Table 2.2: Determination of single extraction procedure for analysis of trace metals in soil and sediments

Extractant	**Metals**	**References**
0.01 M $CaCl_2$	Cd, Cu, Ni, Zn	Novozamsky *et al*, (1993)
0.11 M acetic acid, 0.11 M citric acid, EDTA, HCl (0.65%)	As, Cd, Pb	Pelfrene *et al*, (2020)
MEHLICH 1, MEHLICH 3, EDTA, DTPA-TEA, NH_4OAC, $CACL_2$	Cd, Pb, Cu, Zn	Zhang *et al*, (2010)
EDTA, acetic acid	Pb, Zn, Ni	Chaudhary *et al*, (2016)
EDTA, $CaCl_2$	Cd	Lu, (2019)
CH_3COOH, Na_2EDTA, $CaCl_2$, NH_4NO_3, deionized water	Al, Fe, K, Mn, Na, S, Si, Cd, Co, Cr, Mo, Ni, Pb, V, and Zn	Milicevic *et al*, (2017)
CH_3COOH, citric acid, AB-DTPA, EDTA+NH_4OAC, HNO_3+$HClO_4$+HF (5:3:5, V/V)	As, Cd, Cr, Cu, Pb, and Zn	Luo *et al*, (2019)
$CaCl_2$, CH_3COONH_4, CH_3COOH, EDTA, HCl + HNO_3 (3:1, V/V)	Ba, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, V, and Zn	Sakan *et al*, (2016)

2.4 POLLUTION ASSESSMENT

To assess trace/heavy metal contamination in soil and water for the study, the following pollution indices were applied: contamination factor (C_f); modified degree of contamination (m-C_d), modified heavy metal index (m-HPI); heavy metal evaluation index (HEI); Nemerov index (NeI); geoaccumulation index (I_{geo}); enrichment factor (EF); and pollution load index (PLI).

2.4.1 Modified heavy metal pollution index (m-HPI)

Maskooni *et al*, (2020) specified a modified heavy metal pollution index (m-HPI) for improved evaluation of water quality for domestic use to improve the drawbacks of heavy metals pollution index and heavy metal index (HEI). m-HPI is calculated as follows:

$$m - HPI = \sum_{i=1}^{n} m - HPI^i \quad (1)$$

Where *n* is the number of metals to be assessed and m-HPI^i is the modified heavy metal pollution index for the *i*th heavy metal, calculated as:

$$m - HPI^i = w_i Q_i \quad (2)$$

Where w_i is the relative weightage factor calculated as:

$$W_i = \frac{W_i}{\sum_{i=1}^{n} W_i} \quad (3)$$

Where W_i is the unit weighting factor calculated as:

$$W_i = \frac{1}{S_i} \quad (4)$$

S_i is the maximum standard value of the *i*th heavy metal. Sub index Q_i for the *i*th heavy metal is quantified as:

$$Q_i = \frac{M_i - S_i}{S_i} \quad (5)$$

M_i represents the analysed concentration of the *i*th heavy metal. Modified heavy metal index can be broken down into metals that meet the maximum allowable value or metals that do not meet the maximum allowable value. The previous m-HPI is referred to as the positive index

(PI) and the latter as the negative index (NI) with U_L being the upper limit of PI. Therefore, water quality according to m-HPI may be grouped as shown in table 2.3.

2.4.2 Heavy metal evaluation index (HEI)

HEI offers a description of water quality regarding heavy metal contamination. HEI is evaluated based on Maximum Allowable Concentration (MAC) using the concentration of the metal of interest (Maskooni *et al*, 2020):

$$HEI = \sum_{i=1}^{n} HEI^{i} \tag{6}$$

HEI^i is the contamination index related to the *i*th heavy metal calculated as:

$$HEI^{i} = \frac{M_i}{H^{i}_{MAC}} \tag{7}$$

The MAC of the *i*th heavy metal is H^i_{mac}. To distinguish the various levels of contamination, this method divides water quality into three groups as seen in table 2.3:

2.4.3 Nemerow Index (NeI)

This approach is a multifactorial and integrated technique, where the index is determined using (Maskooni *et al*, 2020):

$$Nel = \left[\frac{\left\{\left(\frac{M_i}{S_i}\right)^2_{average} + \left(\frac{M_i}{S_i}\right)^2_{max}\right\}}{n}\right]^{\frac{1}{2}} \tag{8}$$

Where $(M_i/S_i)_{average}$ is the mean value for all heavy metals of interest of a sample and $(M_i/S_i)_{max}$ is the maximum value for all metals of interest evaluated in the sample. For water quality purposes the technique has four categories of classification (Table 2.3):

2.4.4 Enrichment factor (EF)

In soil sciences, enrichment factor is used to measure/quantify the accumulation of contaminants in environmental samples such as soil or sediments relative to a user-defined background reference element. Commonly used reference elements are Fe, Al, Ca, Ti, Sc, or Mn and is calculated as follows (Kowalska *et al*, 2018):

$$EF = \frac{\left(\frac{C_x}{C_{ref}}\right)_{environment}}{\left(\frac{M_x}{M_{ref}}\right)_{background}} \quad (9)$$

Where C_x (environment) is the concentration of the target trace metal in the environment, C_{ref} (environment) is the concentration of the reference element of the environment, M_x (background) is the concentration of the trace metal, M_{ref} (background) is the total concentration of the reference element. The implication of a reference element in this index makes it superior (Bern *et al*, 2019). EF values varying around 0.5 and 2 can be considered as environmental inputs, while values exceeding 2 suggest some enrichment corresponding to anthropogenic inputs (Shakeri *et al*, 2014). Soil and sediment quality rating are classified into five categories for this technique (Table 2.3).

2.4.5 Geo-accumulation index (I_{geo})

Geoaccumulation Index (I_{geo}) is a commonly utilized index for evaluating the degree of contamination for an individual element. This index is based on the correlation between the analysed metal concentration and its reference value (Kim *et al*, 2018). The reference values used were previously reported by Matong *et al*, (2016) and is calculated as follows:

$$I_{geo} = \log_2\left(\frac{C_n}{1.5B_n}\right) \quad (10)$$

C_n is the analysed concentration of the trace/heavy metal and B_n is the geochemical reference of the metal. Factor 1.5 has been implemented in the formulae because of the potential differences in reference values in the environment for each metal (Kim *et al*, 2018). For soil and sediment quality rating, I_{geo} values are divided into different categories and are listed in table 2.3.

2.4.6 Contamination factor (C*f*)

The assessment of contamination in soil sciences may be evaluated using the contamination factor (C*f*). It is an essential technique for measuring contamination over time (Sivakumar *et al*, 2016). C*f* is a ratio computed by dividing the average concentration of each element by the reference value and is calculated as follows (Shakeri *et al*, 2014).

$$Cf = \frac{C_{heavymetal}}{C_{background}} \quad (11)$$

$C_{heavy\ metal}$ is the concentration of the target metal analysed and $C_{background}$ is the reference concentration of the permissible value. C*f* quality classifications are listed in table 2.3.

2.4.7 Pollution load index (PLI)

This index offers a convenient way to illustrate the degradation of soil quality because of trace/heavy metal accumulation (Kowalska *et al*, 2018). PLI calculates the input of all the metals at a given site and is a statistical average of contamination factor (C*f*) (Holtra & Zamorska, 2020) and is determined by using the formulae given:

$$PLI = \sqrt[n]{(Cf_1 \times Cf_2 \times Cf_3 \times \dots \dots Cf_n)} \quad (12)$$

2.4.8 Modified degree of contamination (m-C_d)

The purpose of m-C_d is to provide an assessment on the overall contamination in soil and sediment surfaces at a given study area (Sivakumar *et al*, 2016) and the equation is given as follows:

$$m - c_d = \frac{\left(\sum_{i=1}^{i=n} Cf\right)}{n} \quad (13)$$

Where *n* is the number of elements evaluated for the *i*th trace/heavy metal and C*f* is the sum of the contamination factor. For soil and water quality statuses the m-C_d is divided into categories (Table 2.3).

2.4.9 Bioavailability of heavy metals

Heavy metal bioavailability can be represented as the extraction or activation rate, as seen:

$$C_A = \left(\frac{C_{Ai}}{C_{Bi}}\right) \times 100 \quad (14)$$

Where C_A is the bioavailability of a selected soil element, C_{Ai} is the analysed concentration of a specific element using single extractant using single extraction, and C_{Bi} is the total concentration of a specific analysed metal using total digestion (Sun *et al*, 2017).

Table 2.3: Quality status for environmental and health parameters

Indices	Value	Quality status	References
	$-1 \leq N I \leq 0$ and $PI = 0$	Excellent	
	$-1 < NI < 0$ and $0 < PI \leq U_L/2$	Very good	
m-HPI	$-1 \leq NI \leq 0$ and $U_L < PI \leq U_L$	Good	Maskooni *et al*, (2020)
	$NI \leq 0$ and $PI > U_L$	Unacceptable	
	HEI < 10	Low	
HEI	HEI= 10 – 20	Medium	Maskooni *et al*, (2020)
	HEI > 20	High	
	NeI < 1	Insignificant contaminated	
NeI	$1 \leq NeI < 2.5$	Slightly contaminated	Maskooni *et al*, (2020)
	$2.5 \leq NeI < 7$	Moderately contaminated	
	$NeI \geq 7$	Heavily contaminated	
	C*f* < 1	Low C*f*	
C*f*	$1 \leq CF < 3$	Moderate C*f*	Sivakumar *et al*, (2016)
	$3 \leq CF < 6$	Considerable C*f*	
	$CF \geq 6$	Very high C*f*	

	$m\text{-}C_d < 1.5$	Nil to a very low degree of contamination	
	$1{,}5 \leq m\text{-}C_d < 2$	Low degree of contamination	
	$2 \leq m\text{-}C_d < 4$	Moderate degree of contamination	
$m\text{-}C_d$	$4 \leq m\text{-}C_d < 8$	High degree of contamination	Sivakumar *et al*, (2016)
	$8 \leq m\text{-}C_d < 16$	Very high degree of contamination	
	$16 \leq m\text{-}C_d < 32$	Extremely high degree of contamination	
	$m\text{-}C_d \geq 32$	Ultra-high degree of contamination	
I_{geo}	<0	Unavailability of contamination	Kim *et al*, (2018)
	0 – 1	From unavailable to fairly contaminated	
	1 – 2	Fairly contaminated	
	2 – 3	From fairly to strongly contaminated	
	3 – 4	Strongly contaminated.	
	4 – 5	From strongly to extremely contaminated	
	>5	Extremely contaminated	
	0 <PLI≤ 1	Uncontaminated	
PLI	1 <PLI≤ 2	Fairly contaminated to uncontaminated	Holtra & Zamorska, (2020)

	2 <PLI≤ 3	Fairly contaminated	
	3 <PLI≤ 4	Fairly to extremely contaminated	
	4 <PLI≤ 5	Extremely contaminated	
	5 <PLI	Excessively contaminated	
E*f*	*Ef* < 2	Insufficiency to minimal enrichment	Shakeri *et al*, (2014)
	E*f* = 2-5	Fair enrichment	
	E*f* = 5-20	Considerable enrichment	
	E*f* = 20-40	Elevated enrichment	
	E*f*> 40	Excessively high enrichment	

2.5 ANALYTICAL TECHNIQUES USED FOR HEAVY METAL ANALYSIS

2.5.1 Atomic Spectroscopy

Atomic spectrometry is a leading method for analysing elements and their compounds in most samples with concentrations down to parts per trillion (Bings *et al*, 2006). Its principal objectives are to achieve the smallest detection limits as possible, remove spectral interferences, and reduces the amount of time spent on sample preparation (Bol'shakov *et al*, 2006). Spectroscopy is a branch in science engaged in the study and calculation of spectra formed during the interaction of matter when it emits electromagnetic radiation (Skoog *et al*, 2007). To gather information about the sample, energy is normally applied to the sample in the form of heat, electrical energy, light, particles, or chemical reactions. The sample analyte is primarily in its lowest energy state (ground state) before it is intensely exposed to high energy levels. After it is exposed to a high energy source some of the analyte species then move off to a higher energy state, called an excited state. Information is then gathered by calculating the electromagnetic radiation released as it returns to its ground state or by measuring the amount of electromagnetic radiation absorbed or scattered due to excitation Fig 2.1 (Skoog *et al*, 2007).

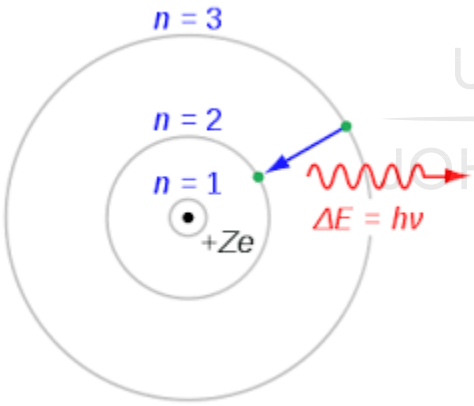

Figure 2.1: Bohr model of an atom (Schirrmacher, 2009).

2.5.1.1 Emission Spectrometry

The emission spectrometry of plasma, arc, and spark has several benefits compared to flame and electrothermal absorption. One of the strengths is the fact that they are less vulnerable to chemical interference due to increased temperatures. Secondly, a satisfactory emission spectrum

for most metals in a single run is noted. Finally, some highly resistant compounds such as oxides of boron, phosphorus, tungsten, uranium, zirconium, and niobium are detectable even at low concentrations due to the powerful plasma source (Skoog et al, 2007).

2.5.1.1.1 Inductively coupled plasma – optical emission spectrometry (ICP – OES)

Optical emission spectrometry is a well-known analytical tool, used extensively over the last four decades. Often the inductively coupled plasma – atomic emission spectrometry (ICP-AES) is called inductively coupled plasma – optical emission spectrometry (ICP - OES) technique (Potts, 1987). The ICP-OES is an effective technique used for the detection of metals in several different sample matrices (Table 2.3) (Ghosh *et al*, 2013). The process is based on the random emission of photons from excited atoms or ions during a radio frequency discharge. Liquid samples are introduced into the instrument directly, unlike solid samples that need to be prepared through extraction or acid digestion before being introduced into the instrument. The sample is then converted into fine droplets and transported into a plasma of about 10 000k where it is vaporized. The analyte atoms or ions are further exposed to greater energy that allows them to be promoted into their excited state. After a while, they relax and come to ground state where they release ray of light energy that has characteristics of each atom and ion. They are then passed through several mirrors and finally to a detector where several photons from each atom or ion detected is directly proportional to its concentration as seen in a schematic diagram in figure 2.2 (Hou *et al*, 2006).

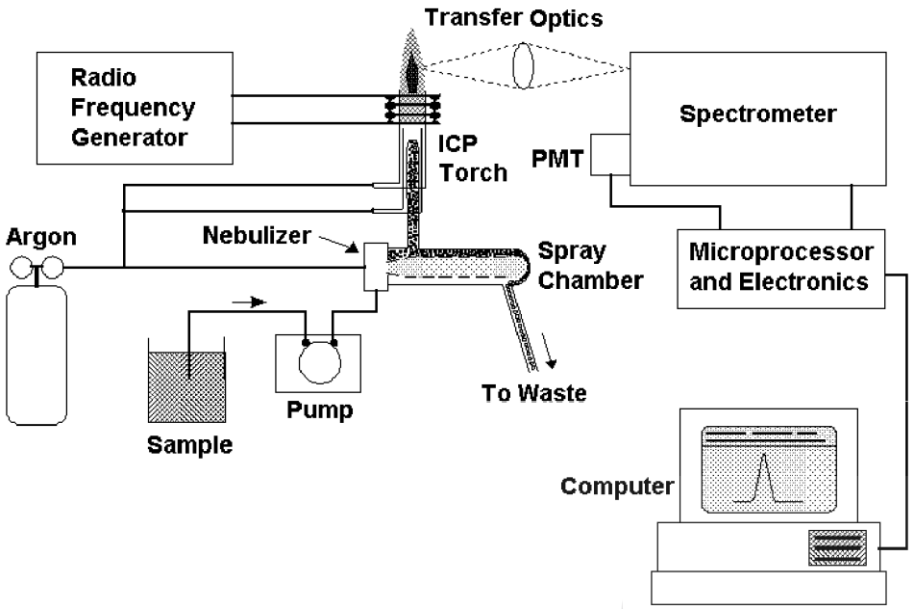

Figure 2.2: Schematic diagram of ICP - OES (Sneddon & Vincent, 2008).

Table 2.4: Types of materials that may be analysed using ICP-OES (Hou et al, 2006).

Categories	**E.g., of samples**
Agriculture and food	Animal tissue, beverages, garlic, pesticides, plant materials, leaves.
Biological and clinical	Blood, faeces, bone, liver, fish.
Geological	Fossils, fossil fuels, coal, sediments, rocks
Environmental and water	Salts, wastewater, groundwater, tap water, sewage sludge.
Metals	Alloys, metals, solders, steel, tin.
Organic	Adhesives, amino acids, cotton, wood, dyes, lubricant, organometallics, oils, polymers.
Other materials	Acids, carbon, catalytic materials, electronics, fibres, paints.

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CHAPTER THREE:
EVALUATION OF SURFACE WATER QUALITY USING VARIOUS INDICES FOR HEAVY METALS IN SASOLBURG

ABSTRACT

Heavy metal pollution in surface waters is a huge problem and a threat to human and environmental health. In this study, heavy metal contamination around selected (Meulsteenpan Lake, Coalplex stream, and Natref stream) in Sasolburg, Free state, South Africa, was assessed. The samples were collected during the dry and wet seasons of 2019 and 2020. Metal concentrations were quantified using inductively coupled plasma-optical emission spectrometer. A compliance study was done using permissible guideline values from the Department of Water Affairs and Forestry (DWAF) of South Africa, World Health Organization (WHO), and Environmental Protection Agency (EPA). Selected physicochemical properties (pH, total dissolved solids (TDS), and electrical conductivity (EC)) of water samples were investigated. The results revealed that the pH of water collected from Meulsteenpan Lake (7.86 – 7.89) and Coalplex stream (7.13 – 7.37) were within DWAF, WHO, and EPA permissible guideline values for domestic and agricultural use. On the other hand, while the pH of water from Natref stream (6.16 – 7.68) was within the permissible guideline values set by DWAF for domestic use but below the permissible guideline values set by WHO and EPA for domestic use and DWAF for agricultural use. The mean TDS and EC values in Meulsteenpan Lake (564 – 980 mg/L) and (1.20 – 2.00 mS) exceeded permissible guideline values set by DWAF and WHO domestic use, Coalplex stream (309 – 326 mg/L) and (0.65 mS), and Natref Stream (269 – 413 mg/L) and (0.56 -0.89 mS) were within the permissible guideline values for DWAF and WHO domestic use. The results obtained for metal concentration revealed higher mean concentrations of Al, Fe, and Mo, Mn in the study area which could pose adverse health risks to aquatic life and humans.

3.1 INTRODUCTION

Water contamination is a huge environmental concern worldwide (Biswas *et al*, 2017). Sasolburg is an industrial town in the Free State province not far from the Vaal triangle and has been pointed out as one of the hotspots of pollution due to anthropogenic activities that released

wastewaters in some of the water streams and pathways (Maponya & Rampedi, 2013). This, in turn, affects the quality of the water bodies which causes the quality to depreciate since large amounts of wastewaters containing different pollutants including trace heavy metals are released and result in the water being unsuitable for domestic use (Saleem *et al*, 2019). Surface water (rivers and streams) has several uses such as consumption (drinking), irrigation, and farming to name a few, and are helpful to humans, animals, and the environment. Therefore, there is a need to monitor heavy metal contamination to prevent environmental deterioration and reduction of biodiversity (Edokpayi *et al*, 2016).

Natural processes and anthropogenic activities are the two main contributors of heavy metals into the environment. Weathering and atmospheric deposition are regarded as natural processes, while industrial waste and sewage are categorized as anthropogenic activities (Yao *et al*, 2014). Industries like mining, mineral processing, agricultural activities, fossil fuel processing, plating, battery productions, smelting, and paint industries are responsible for the introduction of heavy metals into water systems (Nyamukamba *et al*, 2019). Unfavorable effects are experienced when concentrations of heavy metals exceed environmental water guidelines. The use of contaminated water for agricultural and domestic purposes could be harmful to human and animal health (Banzi *et al*, 2015). Although some of these heavy metals (cadmium, mercury, and lead) are toxic even at trace levels, some are essential (such as copper, iron, cobalt, and zinc, among others) to the biological system of humans and animals (Obasi, 2020).

Accumulation of metals in the environment depends on a variety of variables, such as pH of surface water, metal concentration, anthropogenic sources, and other surface water factors. Heavy metal exposure remains a major concern, particularly in undeveloped countries, despite their awareness to humans and the environment (Edokpayi *et al*, 2016). Hence, there is a need to assess the water quality and contamination of surface waters in Sasolburg, which is often used by residents of surrounding areas to meet domestic and agricultural needs. Therefore, the aim of the study was to assess the status of water quality from surface waters and quantify heavy metal concentration and determine possible health risks due to exposure to heavy metals.

3.2 STUDY AREA

The study was conducted in the Meulsteenpan Lake (26.8692° S, 27.8806° E), Coalplex stream (26°49'41.4"S 27°53'45.6"E), and Natref stream (26°48'18.7"S 27°52'33.9"E) in the north of the

Free State province of South Africa in Sasolburg an industrial town which is sub-divided into Sasolburg, Vaalpark and Zamdela (Africa and State, 2020). Sasolburg is located on the east side of Taaibosspruit and adjacent to that stream are industrial areas in the Vaal Triangle. Residential areas are south of the stream and farming practices are to the west (De Klerk, 2010). Weather patterns from this region can differ significantly from west to east. The highest temperatures are mainly experienced in January and temperatures that are lower are experienced in July (DWAF, 2002). In the spring and summer seasons (Oct-Apr) is when there are extremely high volumes of rainfall experienced, although December and January are the peak months for rainfall (DWAF, 2002). Average rainfall per year ranges around 600 mm and 800 mm, with a likelihood for evaporation between 1 300 mm to 1 700 mm annually (DWAF, 2003). Surface water from this region is used for domestic needs, agriculture, and urban use. Research has shown that the standard of surface water is of high quality, but due to anthropogenic activities, chemical reactions from industrial activities have decreased the standard of these waters (De Klerk, 2010).

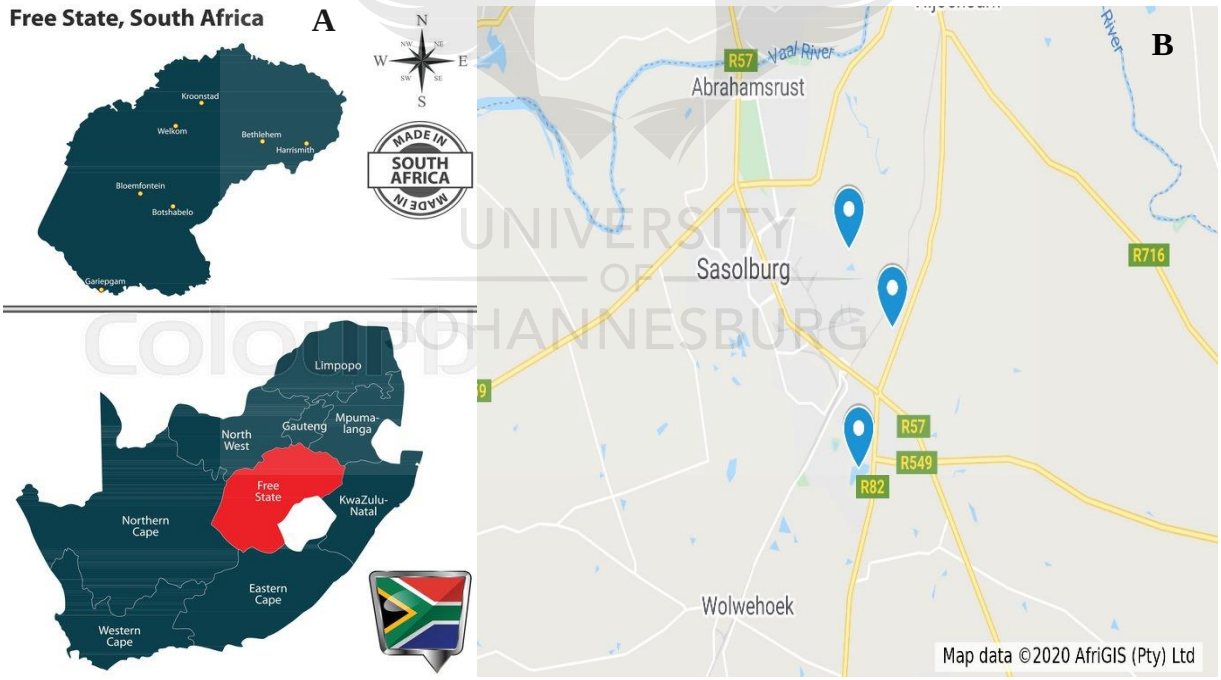

Figure 2.3: Map of A) South African provinces (Vector map of Free State province, 2020 and B) sampling area (Google Maps)

3.3 EXPERIMENTAL

3.3.1 Sample collection

Water samples were collected from selected streams Meulsteenpan Lake, Coalplex stream, and Natref stream. The procedure for sampling was carried out in the dry and wet seasons of 2019 and 2020. In the process of sampling, 1L polyethylene bottles were cleaned with detergent and rinsed with water until they were detergent-free. In the field, the bottles were initially rinsed with water from the selected streams before collection. Samples were collected in duplicate for each sampling site and were collected by dipping the polyethylene bottles into water surface streams with an open-end faced to allow the water to flow in for each sample. Samples were placed in an icebox and transported to the laboratory for further analysis and were preserved by the addition of a few drops of nitric acid (HNO_3) and stored in a ± 4 °C refrigerator before analysis.

3.3.2 Sample analysis

Physicochemical parameters (pH, EC, TDS) were measured. Electrical conductivity (EC) and total dissolved solids (TDS) were measured using digital meters (Ohaus, Conductivity pen meter ST20C-B) and (Ohaus ST20T-B TDS pen meter with temperature) while pH was also measured by using pH meter (Ohaus, ST20 pH pen meter). In the laboratory, samples were filtered with Millipore syringe filter papers (0.45µm). The concentration of heavy metals was analyzed by using the inductively coupled plasma – optical emission spectrometry (ICP-OES) (iCAP 6500 Duo, Thermo Scientific, UK). Samples were analyzed in batches, which consisted of a procedural blank. Quality standards were used to assess each calibration curve before the bundle of samples were analyzed. Water samples were initially filtered using 0.45 µm syringe polyvinylidene difluoride membrane filters and elements analyzed were Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V. The precision of the analysis was carried out in a triplicate reading for each sample.

3.4 RESULTS AND DISCUSSION

3.4.1 Physiochemical parameters

The pH has an influence on the toxicity of heavy metals, non-metallic ions, and vital metals for metabolic activities in humans. Metals like aluminum, cadmium, cobalt, copper, manganese, nickel, and lead are most likely to be unfriendly to the environment as a result of low pH levels in water (DWAF, 1996). Table 3.1-3.3 represents the minimum, maximum, and arithmetic mean pH values of surface water collected from Meulsteenpan Lake, Coalplex stream, and Natref stream during the dry and wet season. pH mean values for Meulsteenpan Lake, Coalplex stream, and Natref stream for the dry season were 7.86, 7.37, and 7.68 while for the wet season they were 7.89, 7.13, and 6.17, respectively. These results showed that the pH values from all three sampling sites in the dry season were within the neutral range and within the permissible guideline values by DWAF, WHO, and EPA. While during the wet season it was noticed that the average pH for Natref stream was low and below the permissible guideline values for DWAF agricultural use, WHO, and EPA. The highly alkaline and acidic conditions were recorded to be 9.49 for Meulsteenpan Lake in the dry season and 4.12 for Natref stream in the wet season. Alkaline water is considered safe for drinking but has adverse side effects such as reducing the acidity found in the stomach which helps remove or destroy bacteria and other unwanted organisms from the bloodstream (Healthline, 2020). For majority of aquatic life, an acceptable pH range of 6.5 - 8.5 is comfortable, with the acid and alkalinity death levels at pH 4 and 11 (Boyd, 2015).

Total dissolved solids (TDS) are mainly made up of inorganic salts dissolved in water. Ions such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, Cl^{-}, and HCO_3^{-} are found in TDS at different concentrations in waters (Baker et al., 2015). Table 3.1-3.3 represents the average TDS concentrations from sampling sites for both dry and wet seasons. The mean concentrations of Meulsteenpan Lake throughout the study were the highest. In the dry and wet seasons, the average TDS concentration for each sampling area were 796 mg/L & 980 mg/L for Meulsteenpan Lake, 324 mg/L & 326 mg/L for Coalplex stream and 269 mg/L & 413 mg/L for Natref stream. Mean concentration from Coalplex and Natref streams were both below the maximum permissible guideline value of (<450 mg/L) for DWAF domestic use and (<600 mg/L) for WHO domestic use, while Meulsteenpan Lake recorded mean levels above the maximum permissible guideline

value set by DWAF and WHO for domestic use for both dry and wet seasons. “Maximum” analyzed values in the dry and wet season for Meulsteenpan Lake were 796 mg/L and 1303 mg/L which are extremely beyond the permissible guideline value for DWAF domestic use and WHO domestic use. TDS mean concentrations of these surface waters from all sampling/study areas for DWAF agricultural use (<40 mg/L) were above the recommended guideline use in the entire study period and may not be considered as irrigation water for agricultural purposes. Detrimental health effects on both human and animals are experienced in water with high TDS due to its adverse effect on feed intake, absorption and its utilization (Sharma et al, 2017).

Electrical current in water is carried out by charged ions. Therefore, the greater the conductivity the greater the concentration of dissolved solids in most water (Li and Liu, 2019). High EC values indicate a significant number of ionic compounds in water (Kabir *et al*, 2002). Conductivity concentrations for each sampling site are listed in Tables 3.1-3.3. Mean EC levels for Meulsteenpan Lake in the dry and wet seasons were 1.20 mS/m and 2.00 mS/m, while for Coalplex stream they were 0.65 mS/m for both dry and wet seasons, and for Natref stream they were 0.56 mS/m for the dry season and 0.89 mS/m for the wet season. The highest EC mean concentrations recorded were in the wet season, with Meulsteenpan Lake having concentrations for both dry and wet seasons above the DWAF permissible guideline values for domestic use (<0.7 mS/m). Coalplex stream recorded average EC levels below the permissible guideline value for both DWAF domestic use (<0.7 mS/m) and WHO domestic use (<2.5 mS/m), while Natref stream had an average EC value for the dry season below the permissible guideline value for both DWAF domestic use (<0.7 mS/m) and WHO domestic use (<2.5 mS/m), with the wet season having an average EC concentration above the DWAF domestic use (<0.7 mS/m) and below the recommended allowable value for WHO domestic use (<2.5 mS/m). In literature mentioned by Edokpayi *et al*, (2017) a study analyzed higher EC concentrations in the wet season which might be caused by precipitation washing off ionic materials from soils into surface waters.

Table 3.1: Average physiochemical parameters (pH, TDS, EC) for Meulsteenpan Lake and permissible water standards

Meulsteenpan Lake							**Permissible water standards**			
	Dry			Wet			DWAF (Domestic use)	DWAF (Agricultural use)	WHO (Domestic use)	EPA (Domestic use)
	Min	Max	Mean	Min	Max	Mean				
pH	6.80	9.49	7.86	7.16	8.93	7.89	6.0 – 9.0	6.5 – 8.4	6.5 – 9.5	6.5 – 9.5
TDS (mg/L)	412	796	564	739	1303	980	450	40	600	-
EC (mS/m)	0.88	1.62	1.20	1.46	2.65	2.00	0.7	-	-	2.5

- = not available

Table 3.2: Average physiochemical parameters (pH, TDS, EC) for Coalplex and permissible water standards

Coalplex stream							**Permissible water standards**			
	Dry			Wet			DWAF (Domestic use)	DWAF (Agricultural use)	WHO (Domestic use)	EPA (Domestic use)
	Min	Max	Mean	Min	Max	Mean				
pH	7.09	7.63	7.37	6.63	7.48	7.13	6. 0 – 9.0	6.5 – 8.4	6.5 – 9.5	6.5 – 9.5
TDS (mg/L)	285	324	309	287	361	326	450	40	600	-
EC (mS/m)	0.62	0.68	0.65	0.54	0.72	0.65	0.7	-	-	2.5

- = not available

Table 3.3: Average physiochemical parameters (pH, TDS, EC) for Natref and permissible water standards

Natref stream							**Permissible water standards**			
	Dry			Wet			DWAF (Domestic use)	DWAF (Agricultural use)	WHO (Domestic use)	EPA (Domestic use)
	Min	Max	Mean	Min	Max	Mean				
pH	7.02	8.21	7.68	4.12	7.91	6.16	6. 0 – 9.0	6.5 – 8.4	6.5 – 9.5	6.5 – 9.5
TDS (mg/L)	161	429	269	221	654	413	450	40	600	-
EC (mS)	0.27	0.96	0.56	0.47	1.47	0.89	0.7	-	-	2.5

- = not available

3.4.2. Heavy metal concentrations in surface water

3.4.2.1 Arsenic (As)

The mean concentrations for arsenic ranged from 0.62 – 3.63 ug/L, 4.30 – 10.22 ug/L, and 2.18 – 10.64 ug/L in Meulsteenpan Lake, Coalplex stream, and Natref stream, respectively (Table 3.4-3.6). The concentration of As in all water samples collected from the three study areas was below the guideline values of < 100 ug/L donated by DWAF for agricultural use. For Meulsteenpan Lake, the dry season had low concentrations for arsenic and these levels complied with the permissible guideline values set by DWAF, WHO, and EPA for domestic use. In contrast, the wet season had higher concentrations (maximum of 12.62 ug/L) and they exceeded the permissible guideline values for domestic use set by DWAF, WHO, and EPA. The dry season for Coalplex stream had higher concentrations compared to the wet season of arsenic. The mean concentrations of arsenic in the dry season was 10.22 ug/L with a maximum of 30.28 ug/L which both exceeded the domestic guideline limits by DWAF, WHO, and EPA. Natref stream recorded high concentration in dry seasons. The average mean of 10.64 ug/L and maximum concentration of 31.92 ug/L were above the guideline values for domestic use donated by DWAF, WHO, and EPA. High concentrations of As from these two sites originates from coal combustion and pesticide application (Atsdr, 2021), since both sites are located next to a coal mine and agricultural site high levels of As may be expected. Elevated concentrations of arsenic in water have detrimental effects on the environment and humans. Arsenic has no significant contribution to the metabolic system of the human body and studies have shown that multiple cancers are associated with high intake and accumulation of arsenic through the indigestion of the metal via drinking water (WHO, 2017).

3.4.2.2 Cadmium (Cd)

The mean concentrations of Cd in water samples collected from Meulsteenpan Lake, Coalplex stream, and Natref stream ranged from 0.36 – 1.03 ug/L, 0.056 – 0.21 ug/L, 0.073 – 0.77 ug/L, respectively (Table 3.4-3.6). The detected concentrations for cadmium in all three sites were below the permissible guideline limits for WHO, DWAF, and EPA for domestic and agricultural use. Even though the levels were generally below the permissible guideline values, it was

observed that during the dry season relatively high concentrations of Cd were observed at Meulsteenpan Lake and Natref stream. Excessive exposure to cadmium has several acute and chronic effects on the human body. When the metal enters the human biological system, it accumulates in the kidney and this may lead to the development of kidney stones. Other critical effects of cadmium exposure are the ability to interrupt body metabolism and high exposure to the metal may cause cancer (WHO, 2003). Although the concentrations of Cd were detected in trace levels, long-term exposure affects vital organs such as the liver and kidney cortex because about where 30–60% of the ingested Cd is deposited in these organs (Samples *et al*, 2017). Also, the presence of Cd in the environment is detrimental even at low concentrations because it tends to reduce the uptake of zinc by plants and animals (Cheng *et al*, 2018; Driessnack *et al*, 2017; Rizwan *et al*, 2019).

3.4.2.3 Cobalt (Co)

Mean concentration for dry and wet season ranged from 2.61 – 9.01 ug/L, 2.61 – 9.01 ug/L, and 6.72 – 8.78 ug/L for water samples collected from Meulsteenpan Lake, Coalplex stream, and Natref stream, respectively (Table 3.4-3.6). No domestic permissible guidelines values were reported for cobalt by DWAF, WHO, and EPA for domestic use. For DWAF agricultural use the average concentrations in all three sites during the dry and wet seasons were below the guideline value < 50 ug/L. Cobalt compounds are easily dissolvable and far more toxic than those that are difficult to dissolve in water. When the metal enters the human body, it spreads to vital organs like the liver, kidney, and bones (Nyamukamba et al, 2019).

3.4.2.4 Chromium (Cr)

In this study the average concentration of Cr in surface water samples ranged from 2.17 - 3.93 ug/L, 0.30 – 1.29 ug/L, and 1.86 – 2.07 ug/L in Meulsteenpan Lake, Coalplex stream, and Natref stream, respectively (Table 3.4-3.6). The mean average concentrations were below the permissible guideline values set by DWAF, WHO, and EPA < 50 ug/L for domestic use and < 100 ug/L for DWAF agricultural use. Higher mean concentrations for Meulsteenpan Lake were detected in the wet season, while for Coalplex stream and Natref stream higher concentrations were witnessed in the dry season. The high concentration of Cr during the wet season could be due to the leaching of metals from the soil to surface waters (Obasohan, 2008). It should be

noted that Coalplex and Natref streams are located close to fossil fuel operations that might lead to high Cr levels compared to Meulsteenpan Lake. The contribution of industries on the concentration of Cr in surface water has been reported in Greece (Kazakis *et al*, 2017), India (Manoj *et al*, 2020), and South Africa (Coetzee *et al*, 2020).

Table 3.4: Minimum, maximum, and mean concentrations of surface water from Meulsteenpan Lake (ug/L)

	Seasonal variation						Permissible water standards			
	Dry			Wet			DWAF (Domestic use)	DWAF (Agricultural use)	WHO (Domestic use)	EPA (Domestic use)
	Min	Max	Mean	Min	Max	Mean				
Al	21.7	34.9	27.3	17.7	1381	363	150	5000	N.A.	200
As	<DL	1.85	0.62	<DL	12.68	3.63	10	100	10	10
Cd	0.05	2.27	1.03	0.12	0.78	0.36	5	10	3	5
Co	0.59	23.93	9.01	0.23	5.47	2.61	N.A.	50	N.A.	N.A.
Cr	0.73	3.68	2.17	0.73	10.12	3.93	50	100	50	50
Cu	<DL	17.32	8.25	1.59	7.43	3.91	1000	200	2000	2000
Fe	<DL	<DL	<DL	<DL	6028	2078	100	5000	N.A.	200
Mn	0.93	18.4	10.9	0.93	1093	569	50	50	N.A.	50
Mo	3.53	13.53	7.12	0.68	13.53	7.59	N.A.	10	N.A.	N.A.
Ni	<DL	2.05	0.68	<DL	0.35	0.088	N.A.	200	70	20
Pb	1.38	3.48	2.66	0.22	5.35	2.15	10	200	10	10
Sb	<DL	1.7	1.05	1.65	7.78	4.57	N.A.	N.A.	20	5
Se	<DL	28.1	15.53	2.6	28.12	15.8	20	20	40	10
Sr	145	291	222	291	492	360	N.A.	N.A.	N.A.	N.A.
Tl	6.12	20.1	14.0	<DL	18.5	11.4	N.A.	N.A.	N.A.	N.A.
V	<DL	22.0	14.2	3.47	43.5	22.0	100	100	N.A.	N.A.

N.A = Not available, <DL = below detection limit

3.4.3.5 Copper (Cu)

Copper is an essential metal for metabolic use and is also considered a contaminant in drinking water. The primary source of copper intake is food and water (Obasi & Akudinobi, 2020). The results obtained in this study revealed that water samples from Meulsteenpan Lake, Coalplex stream, and Natref stream contained an average Cu concentration ranging from 3.91 – 8.25 ug/L 2.42 – 4.61 ug/L, and 5.97 – 7.38 ug/L, respectively (Table 3.4-3.6). The highest maximum concentrations (17.31 – 18.00 ug/L) from all three sites were recorded from Natref stream for both dry and wet seasons. Mean concentrations analyzed on all sites were below the guideline value < 1000 ug/L DWAF, < 2000 ug/L for WHO, and EPA for domestic use, and < 200 ug/L DWAF agricultural use. The threshold for the effects of copper in drinking water on the gastrointestinal tract has been delineated in recent research, but there is still some confusion as to the long term effects of copper on susceptible populations, such as Wilson disease gene carriers and other copper homeostasis metabolic disorders (WHO, 2017).

3.4.2.6 Iron (Fe)

The mean concentrations of Fe throughout the assessment for Meulsteenpan Lake, Coalplex stream, and Natref stream ranged from 0 - 2078 ug/L, 52.0 – 219 ug/L, and 4.96 – 115 ug/L, respectively (Table 3.4-3.6). Permissible guideline values for WHO were not documented for Fe. High mean concentrations in the wet season on all three study areas were recorded. No iron concentrations were detected in Meulsteenpan Lake in the dry season, while in the wet season a mean concentration of 2078 ug/L and maximum amount up to 6028 ug/L were detected which exceeded the guideline values set by DWAF and EPA for domestic use, and DWAF for agricultural use. The high concentration levels from these surface waters may be due to waste released from industrial factories or a non-point source from rainwater runoff. At concentrations greater than 300 ug/L, iron damages laundry and plumbing components (WHO, 2017).

3.4.2.7 Manganese (Mn)

The results obtained in this study shows that the mean concentrations of manganese in Meulsteenpan Lake ranged from 10.93 – 567.85 ug/L, while average concentrations ranging

from 34.87 – 272 ug/L, and 40.74 – 60.41 ug/L were detected in the water samples collected from Coalplex and Natref streams (Table 3.4-3.6). The permissible guideline values for WHO domestic use were not available. Meulsteenpan Lake recorded the highest average concentrations in the wet season with a maximum of 1093 ug/L. In the wet season high mean concentration of manganese was analyzed from all three sites which exceeded the permissible guideline limits donated by < 50 ug/L for DWAF and EPA domestic use and < 20 ug/L DWAF agricultural use. Although manganese is an essential element, excessive exposure to the metal can be harmful to humans and may cause neurotoxicity. Manganism is a neurological disease caused by high exposure to manganese with similar symptoms as Parkinson's disease such as gait disorder, action tremor, rigidity, bradykinesia, memory dysfunction, and mood disorder (Obasi & Akudinobi, 2020).

3.4.2.8 Molybdenum (Mo)

Table 3.4-3.6 shows that the average concentrations of Mo in surface water collected during the wet and dry seasons were found to be 7.12 – 7.59 ug/L, 208 – 341 ug/L, and 3.33 – 3.38 ug/L for Meulsteenpan Lake, Coalplex stream, and Coalplex stream. There are no permissible guideline values for molybdenum set by DWAF, WHO, and EPA for domestic use. Meulsteenpan Lake had a maximum of 13.5 ug/L in both dry and wet seasons which exceeded the permissible guideline value of < 10 ug/L set by DWAF for agricultural use. Coalplex stream had high concentrations of Mo for both dry (minimum and maximum of 244 ug/L and 397 ug/L) and wet (32.5 ug/L and 470 ug/L) seasons, which exceeded the permissible guideline value set by DWAF for agricultural use. The concentration Mo at Natref stream was within the DWAF permissible guideline value < 10 ug/L for agricultural use. High concentration experienced at Coalplex stream owes it to the stream being near mining facilities. WHO (2017) reported that concentration levels greater than 200 ug/L may be recorded in areas located next to mines.

Table 3.5: Minimum, Maximum, and Mean concentrations of surface water from Coalplex Stream (ug/L)

	Seasonal variation						**Permissible water standards**			
	Dry			**Wet**			**DWAF (Domestic use)**	**DWAF (Agricultural use)**	**WHO (Domestic use)**	**EPA (Domestic use)**
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**				
Al	14.1	51.3	27.3	44.1	143	94.4	150	5000	N.A.	200
As	<DL	30.28	10.2	1.72	7.10	4.30	10	100	10	10
Cd	<DL	0.13	0.056	0.1	0.38	0.21	5	10	3	5
Co	<DL	4.68	2.03	1.33	36.6	10.8	N.A.	50	N.A.	N.A.
Cr	<DL	3.88	1.29	<DL	1.13	0.30	50	100	50	50
Cu	<DL	8.75	4.61	1.56	4.00	2.42	1000	200	2000	2000
Fe	<DL	96.8	52.0	<DL	550	279	100	5000	N.A.	200
Mn	2.93	51.9	34.9	0.4	675	272	50	50	N.A.	50
Mo	244	397	341	32.5	470	208	N.A.	10	N.A.	N.A.
Ni	<DL	<DL	<DL	<DL	<DL	<DL	N.A.	200	70	20
Pb	<DL	1.57	0.87	2.15	6.95	3.81	10	200	10	10
Sb	<DL	4.62	2.27	1.57	7.84	3.49	N.A.	N.A.	20	5
Se	<DL	20.7	7.47	<DL	22.0	6.27	20	20	40	10
Sr	201	301	240	146	252	201	N.A.	N.A.	N.A.	N.A.
Tl	4.83	28.9	20.8	<DL	15.7	4.48	N.A.	N.A.	N.A.	N.A.
V	<DL	24.7	15.0	<DL	20.3	9.22	100	100	N.A.	N.A.

N.A = Not available, <DL = below detection limit

3.4.2.9 Nickel (Ni)

As seen in Table 3.4-3.6, the mean concentrations of nickel found in Meulsteenpan Lake were between 0.088 and 0.67 ug/L. On the other hand, the average concentration of Ni in surface water from Natref stream was 0 – 0.67 ug/L in Table 3.4-3.6. As seen in Table 3.4-3.6 it shows that in Coalplex stream Ni was not detected. There were no guideline values set by DWAF domestic use. Mean concentrations on all three study areas were below the permissible guideline values of < 70 ug/L and < 20 ug/L for WHO and EPA domestic use, and < 200 ug/L DWAF agricultural use. Nickel is a very harmful metal and is considered carcinogenic by IARC. For non-smokers or persons exposed to it occupationally, its main source of exposure is food (WHO, 2017).

3.4.2.10 Lead (Pb)

The mean concentration of Pb found in Table 3.4 – 3.6 from Meulsteenpan Lake, Coalplex stream and Natref stream ranged from 2.15 – 2.66 ug/L, 0.87 – 3.81 ug/L, and 2.15 – 3.21 ug/L for both dry and wet seasons, respectively. All water samples throughout the study obtained mean concentrations below the standard guideline values of < 10 ug/L set by DWAF, WHO, and EPA for domestic use, and < 200 ug/L set by DWAF for agricultural water use. Lead is a cumulative metal and exposure to Pb is extremely harmful both on humans and the environment and is of concern to many parts of the world. Symptoms associated with Pb poisoning are headache, irritability, and abdominal pain. When higher concentrations of Pb are found they may cause kidney dysfunction and brain damage (Obasi & Akudinobi, 2020).

3.4.2.11 Antimony (Sb)

Exposure to antimony is primarily through food and water even though it has minimal contribution compared to occupational exposure. When combined with lead, copper, and tin the metal forms very hard alloys (WHO, 2017). Mean concentrations for Sb detected in Meulsteenpan Lake ranged from 1.05 – 4.57 ug/L while in Coalplex stream they ranged 1.57 – 3.49 ug/L (Table 3.4-3.6). Average concentrations ranging from 0.87 – 1.52 ug/L were detected at Natref stream (Table 3.4-3.6). Standard guideline values set by DWAF were not available for Sb for both domestic and agricultural water use. In Meulsteenpan Lake the mean concentration

levels were below the standard guideline value < 20 ug/L and < 5 ug/L set by WHO and EPA. Coalplex stream had mean concentrations below the permissible guideline value < 20ug/L and < 5 ug/L set by WHO and EPA, except for the wet season when a maximum of 7.84 ug/L was recorded and was above the permissible guideline value set by EPA < 5 ug/L. Natref stream had mean concentration levels below the permissible guideline value of < 20 ug/L and < 5 ug/L set by WHO and EPA.

3.4.2.12 Selenium (Se)

The mean concentrations of selenium in this study were found to be 15.5 – 15.6 ug/L (Meulsteenpan Lake), 6.27 – 7.47 ug/L (Coalplex stream), and 6.47 – 8.27 ug/L (Natref stream) for both dry and wet seasons, respectively (Table 3.4 – 3.6). As seen in Table 3.4 - 3.6, a maximum of Se concentration in Meulsteenpan Lake was found to be 28.1 ug/L for both dry and wet seasons, while in Coalplex stream up to 22.0 ug/L Se was detected. Natref stream was found to contain a maximum ranging between 18.5 ug/L and 23.37 ug/L for both dry and wet seasons. The concentrations of Se in all three sites were above the permissible guideline values of < 20 ug/L and < 10 ug/L set by DWAF and EPA domestic use, and < 20 ug/L set by DWAF agricultural use expect for Natref stream during the dry season which only exceeded the guideline value set by EPA. Se concentrations in all sampling sites were below the permissible guideline value of < 40 ug/L set by WHO. High concentrations of selenium in these surface waters are caused by the mobility of selenium compounds dissolved in wastewater released by industries. Surface waters may also receive large concentrations of selenium from the atmosphere through the rain (Engwa et al, 2018; Garvey et al, 2013). Exposure to high concentrations of selenium may lead to vomiting, nail change, loss of energy, and irritability (WHO, 2017).

3.4.2.13 Vanadium (V)

In this study the mean concentrations for vanadium in Meulsteenpan Lake, Coalplex stream, and Natref stream ranged from 14.2 – 22.0 ug/L, 9.22 – 14.96 ug/L, and 13.1 – 14.6 ug/L for both dry and wet seasons, respectively (Table 3.4-3.6). The permissible guideline values for vanadium were not available for WHO and EPA. The mean concentration detected in the water samples for vanadium were all below the standard permissible guideline < 100 ug/L set by DWAF for

domestic and agricultural use. Exposure to high concentrations of vanadium may cause respiratory disorders, paralysis, and negative effects on the liver and kidney (Vanadium (V) - Chemical properties, Health and Environmental effects, 2020).

Table 3.6: Minimum, Maximum, and Mean concentrations of surface water from Natref Stream (ug/L)

	Seasonal variation						**Permissible water standards**			
	Dry			**Wet**			**DWAF (Domestic use)**	**DWAF (Agricultural use)**	**WHO (Domestic use)**	**EPA (Domestic use)**
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**				
Al	21.7	58.9	41.1	10.6	495	145	150	5000	N.A.	200
As	<DL	31.9	10.6	<DL	6.41	2.18	10	100	10	10
Cd	<DL	2.27	0.77	<DL	0.17	0.073	5	10	3	5
Co	<DL	23.9	8.78	0.4	22.4	6.72	N.A.	50	N.A.	N.A.
Cr	<DL	4.13	2.07	0.33	3.25	1.86	50	100	50	50
Cu	<DL	17.3	5.97	1.55	18.0	7.58	1000	200	2000	2000
Fe	<DL	14.9	4.96	<DL	426	115	100	5000	N.A.	200
Mn	18.4	75.3	40.7	0.27	227	60.4	50	50	N.A.	50
Mo	2.6	4.28	3.33	0.45	6.00	3.38	N.A.	10	N.A.	N.A.
Ni	<DL	2.02	0.67	<DL	<DL	<DL	N.A.	200	70	20
Pb	1.00	5.50	3.21	0.20	5.25	2.15	10	200	10	10
Sb	0.30	1.45	0.87	<DL	2.5	1.52	N.A.	N.A.	20	5
Se	0.15	18.5	8.27	<DL	23.4	6.47	20	20	40	10
Sr	145.	599	317	168	332	272	N.A.	N.A.	N.A.	N.A.
Tl	3.73	30.5	18.1	4.00	18.4	9.59	N.A.	N.A.	N.A.	N.A.
V	<DL	22.0	14.6	3.00	20.3	13.1	100	100	N.A.	N.A.

N.A = Not available, <DL = below detection limit

3.4.3 Pollution assessment on surface water

3.4.3.1 Contamination factor (Cf)

Figure 3.1-3.3 (Table 3A-1 – 3A-3) presents the values contamination factors (C*f*) for all the metals in surface water collected from Meulsteenpan Lake, Coalplex stream and Natref stream during the dry and we season. The values were calculated using DWAF, WHO, and EPA permissible values for domestic and agricultural uses. The values of C*f* for the investigated metals differ based on the sampling site. For example, the C*f* values for As showed moderate contamination (moderate ($1 \leq Cf \leq 3$) in Natref and Coalplex (Figure 3.1). Suggesting that the source of As in surface water comes from industrial activities. The C*f* values for the rest of the investigated metals displayed low contamination ($Cf < 1$) in all sampling sites except Se in Meulsteenpan Lake (Figure 3.1b).

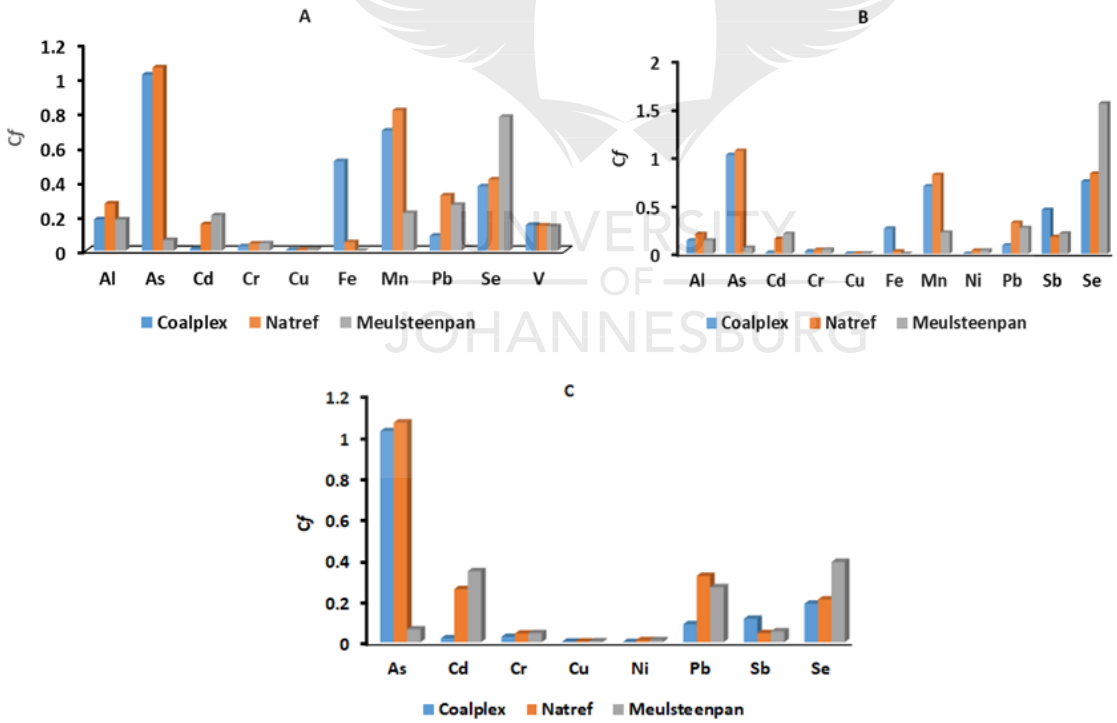

Figure 3.1: Contamination factors (Cf) for all the metals in surface water collected from Meulsteenpan Lake, Coalplex stream and Natref stream during the dry season: Cf values were calculated using a) DWAF, b) EPA and c) WHO permissible values for domestic use.

During the wet season, a significant increase in the contamination was observed for Meulsteenpan Lake. The C*f* for most of the metals was >1 implying that the contamination ranged from moderate to very high (Figure 3.2a-c). As seen in Figure 3.2a$_1$ iron and manganese had very high contamination factor values (C*f* ≥6). These findings suggest that most of the metals leached to the surface water and are not suitable for consumption without treatment.

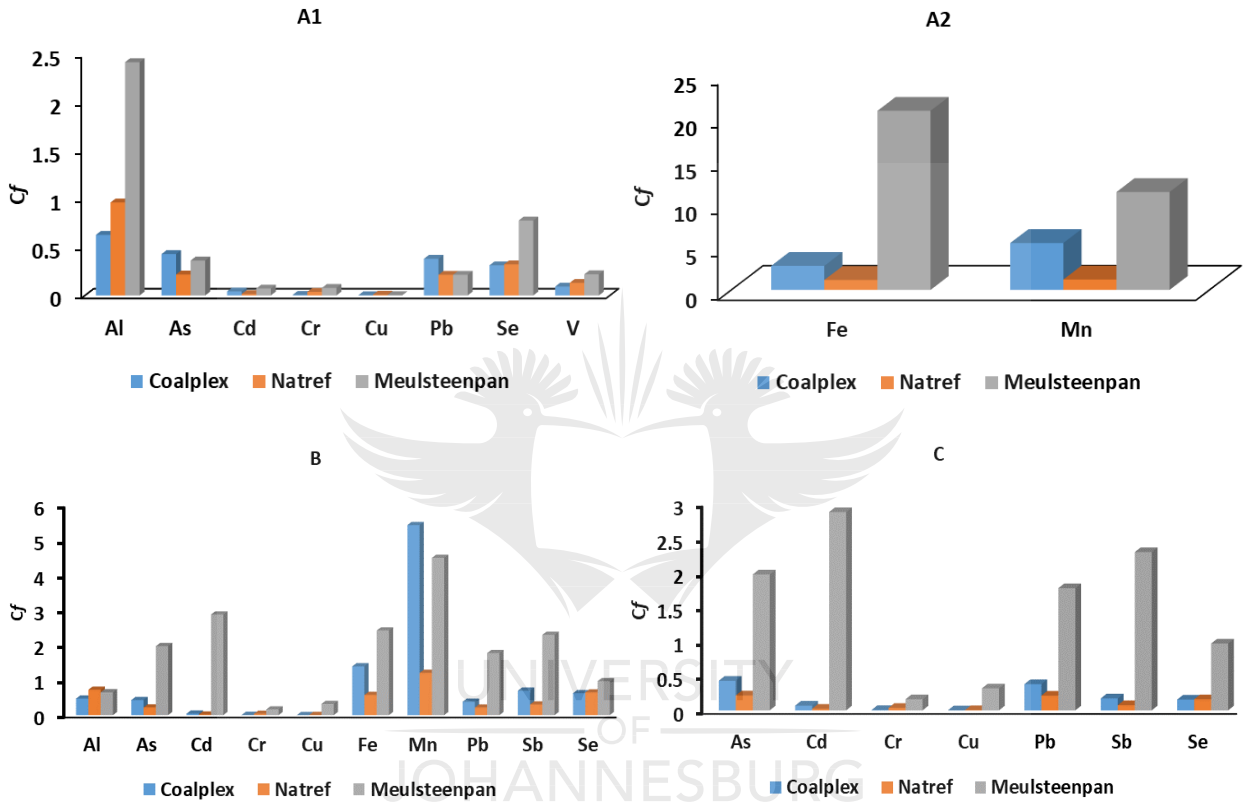

Figure 3.2: Contamination factors (Cf) for all the metals in surface water collected from Meulsteenpan Lake, Coalplex stream and Natref stream during the wet season: Cf values were calculated using a1-2) DWAF, b) EPA and c) WHO permissible values for domestic use.

In Figure 3.3 DWAF permissible guideline values for agricultural use were used to assess the contamination factor for each metal. Figure 3.3 shows a low contamination factor for most metals C*f* <1, except for Mo in Coalplex stream (Figure 3.3 a and b), Mn in all three sampling sites (Figure 3.3). Based on the results, this shows that the study area is highly affected by heavy metal contamination and regular assessment should be followed due to bioaccumulation. This

contamination is due to rainwater wash-off, industrial air pollution, wastewater contamination, and agricultural practices.

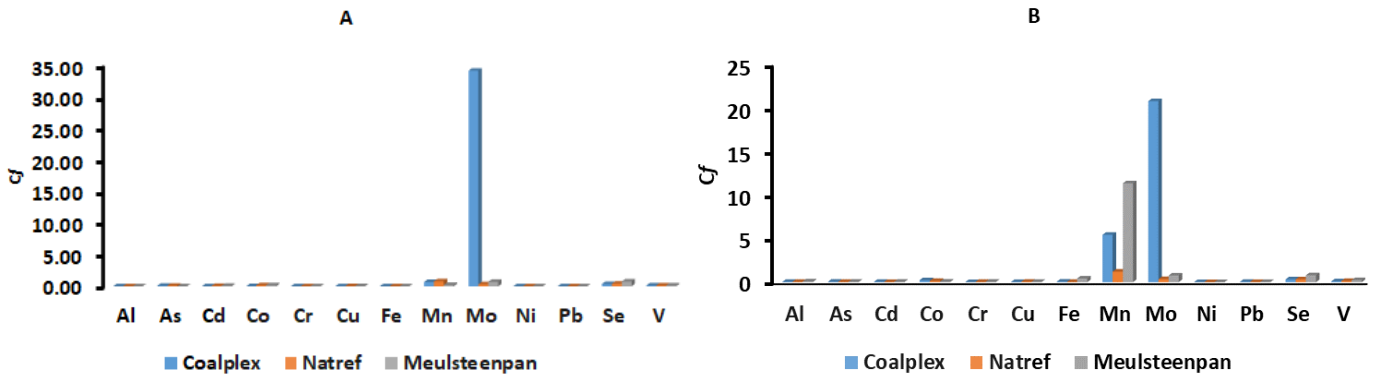

Figure 3.3: Contamination factors (Cf) for all the metals in surface water collected from Meulsteenpan Lake, Coalplex stream and Natref stream during a) dry and b) wet season: Cf values were calculated using DWAF permissible values for agricultural use.

3.4.3.2 Modified degree of contamination (m-C_d)

Results presented in Table 3.7 show the degree of contamination when all metals are taken into consideration. Modified degree of contamination for this study exhibited moderate, low, and nil to a very low degree of contamination as seen in Table 2.3. This also shows that most high contamination was experienced in the wet season. As expected, these results correlated with the contamination factor.

Table 3.7: m-C_d results based on WHO, EPA, and DWAF permissible standards.

	WHO Domestic use	**EPA Domestic use**	**DWAF Domestic use**	**DWAF Agricultural use**
Meulsteenpan Lake (Dry Season)	0.146	0.249	0.191	0.171
Meulsteenpan Lake (Wet Season)	0.175	2.433	3.629	1.061
Coalplex Stream (Dry Season)	0.182	0.130	0.307	2.734
Coalplex Stream (Wet Season)	0.152	0.862	1.012	2.079
Natref Stream (Dr Season)	0.243	0.333	0.328	0.165
Natref Stream (Wet Season)	0.092	0.358	0.427	0.176

3.4.3.3 Modified heavy metal pollution (m-HPI)

Modified heavy metal pollution was used to assess the heavy metal pollution in surface water from the study area in both dry and wet seasons. The values for m-HPI are presented in Table 3.8 and ranged from 0.54 – 10.5. Based on the results obtained only 8.3% of the samples were unacceptable for domestic and agricultural use with 91.7% of the samples ranked as excellent to good in the study area. The highest recorded values were 6.73 and 10.56 at Coalplex stream using DWAF permissible standards set for agricultural use. This high status may be due to the high release of contaminants from industrial wastewater. The results of m-HPI were in line with those reported in literature (Elumalai *et al*, 2017; Vu *et al*, 2017).

Table 3.8: m-HPI results using WHO, EPA, and DWAF permissible standards

	WHO Domestic use m-HPI	**EPA Domestic use m-HPI**	**DWAF Domestic use m-HPI**	**DWAF Agricultural use m-HPI**
Meulsteenpan Lake (Dry Season)	0.7503	0.7897	0.7668	0.6115
Meulsteenpan Lake (Wet Season)	0.8126	0.9089	1.5077	1.1921
Coalplex Stream (Dry Season)	0.8078	0.6314	0.7106	10.4676
Coalplex Stream (Wet Season)	0.8178	0.7144	0.8020	6.7395
Natref Stream (Dry Season)	0.7141	0.6207	0.5416	0.7482
Natref Stream (Wet Season)	0.9067	0.7462	0.7941	0.7877

3.4.3.4 Heavy Metal Evaluation Index (HEI)

Table 3.9 represents HEI results for the study area for the dry and wet seasons. HEI results ranged from 0.74 - 36.3 with seventy-five percent (75%) of the results falling into category one (low <10). Meulsteenpan Lake (wet season) averagely had the highest contamination throughout the entire study period.

Table 3.9: HEI results based on WHO, EPA, and DWAF.

	WHO Domestic use HEI	**EPA Domestic use HEI**	**DWAF Domestic use HEI**	**DWAF Agricultural use HEI**
Meulsteenpan Lake (Dry Season)	1.1693	2.7335	1.9046	2.2236
Meulsteenpan Lake (Wet Season)	1.3976	26.7673	36.2854	13.7972
Coalplex Stream (Dry Season)	1.4560	3.4433	3.0731	35.5471
Coalplex Stream (Wet Season)	1.2195	9.4865	10.1204	27.0285
Natref Stream (Dry Season)	1.9459	3.6631	3.2847	2.1457
Natref Stream (Wet Season)	0.7361	3.9484	4.2735	2.2835

3.4.3.5 Nemerow Index (NeI)

Table 3.10 represents Nemerow index (NeI) results. Results obtained from the study area of all three sites showed that low contamination was experienced throughout the study period. All results were in category one (1) as seen in Table 2.3. Results ranged from 0.01921 – 0.4695.

Table 3.10: NeI results using WHO, EPA, and DWAF permissible

	WHO Domestic use NeI	**EPA Domestic use NeI**	**DWAF Domestic use NeI**	**DWAF Agricultural use NeI**
Meulsteenpan Lake (Dry Season)	0.04716	0.04297	0.07896	0.01345
Meulsteenpan Lake (Wet Season)	0.4695	0.4251	0.7758	0.1299
Coalplex Stream (Dry Season)	0.09626	0.08563	0.1531	0.02424
Coalplex Stream (Wet Season)	0.1295	0.1129	0.1966	0.02854
Natref Stream (Dry Season)	0.06738	0.06139	0.1128	0.01921
Natref Stream (Wet Season)	0.05280	0.07183	0.1279	0.02001

3.5 CONCLUSIONS

In this study, physiochemical parameters (pH, EC, TDS), heavy metals, and pollution assessments were applied to assess the surface water quality/contamination. Physiochemical parameters for the study area were measured and Meulsteenpan Lake having high pH, TDS, and EC as compared to Coalplex stream and Natref stream. The lowest pH results recorded at Natref

stream suggested that the surface water was acidic due to industrial waste and they were found to be above the permissible guideline limits. The heavy metal results revealed that aluminum, iron, molybdenum, and manganese were detected in high concentrations in all sites as compared to other investigated metals. Also, the mean concentrations for the three study areas in all heavy metals exhibited high concentrations especially in the wet season which exceeded the permissible guideline values. Pollution indices showed that the surface water was exposed to heavy metal contamination. The contamination factor (C*f*) revealed the effect of contamination posed by each metal to these surface waters with iron and manganese having very high contamination C*f* >6. For the degree of contamination exhibited moderate, low, and nil to a very low degree of contamination was exhibited. Modified heavy metal pollution index, heavy metal evaluation index, and Nemerow index showed that the majority of the investigated surface water had low contamination. Coalplex stream was found to have a high and unacceptable ranking for m-HPI and HEI. Suggesting that the mining activity around this area has affected the water quality. Based on the evaluation of the results obtained from this study area, these surface waters are highly impacted by anthropogenic activities. Heavy metal pollution indices showed the importance of grouping surface water contamination to heavy metals. This research presented a vital problem and showed that surface waters located next to industrial facilities are under pressure in many parts of the world.

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CHAPTER FOUR:
BIOAVAILABILITY ASSESSMENT OF TRACE/HEAVY METALS USING SINGLE EXTRACTION IN SEDIMENTS FROM SASOLBURG, SOUTH AFRICA.

ABSTRACT

Single extraction method using acetic acid, EDTA, $CaCl_2$, and deionized water as extractants, and pseudo-total digestion (aqua regia) were utilized to determine trace/heavy metals (Al, As, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V) in sediments from South Africa, Free state, Sasolburg. Metal concentrations were quantified by inductively coupled plasma – optical emission spectroscopy (ICP-OES). Ultrasonic bath was used to help assist with the extraction of major and trace metals and decrease the extraction time. Results obtained from Meulsteenpan Lake, Coalplex stream, and Natref stream from single extraction showed that higher extraction was of Al, Fe, and Mn was witnessed compared to other metals in all three sampling sites. EDTA, deionised water, and acetic acid were far more aggressive extractants. The pH of sediments showed that they were slightly acidic. Environmental assessments such as contamination factor, modified degree of contamination, enrichment factor, geo-accumulation index, and pollution load index were used to help evaluate trace/heavy metal contamination.

4.1 INTRODUCTION

Soil and sediment biota is one of the environmental mediums that are commonly at risk of heavy metal contamination (Olaniran *et al.*, 2013). Although metals exist naturally in soil, many factors contribute to the accumulation of these metals which leads to soil/sediment contamination (Takáč *et al.*, 2009). Some of these factors are from anthropogenic activities like the use of fertilizers on soil, mining, and waste from surrounding industrial dumping sites (Zovko *et al.*, 2011). Heavy metal contamination in soil and sediments can have an effect on the bioavailability of metals and as a result several negative effects on humans, plants, and micro-organisms that are habitats in soil (Zovko *et al.*, 2011; Barnett *et al.*, 2003). Metals occur in soil and sediments in their inactive form and their state is affected by changes in physicochemical properties of soil or sediments that may cause them to react with each other, thus affecting the bioavailability and mobility of other metals (Ashraf *et al.*, 2014). Change in chemical properties in soil and sediments may be due to environmental conditions in which have an influence on the toxicity of metals that affect soil microorganisms and plants (Zovko

et al., 2011; Kim *et al.*, 2015; Olaniran *et al.*, 2013). The bioavailability of trace/heavy metals in soil and sediment biota are influenced by physicochemical properties such as pH, type (texture), as well as cation-exchange capacity (CEC) (Zovko *et al.*, 2011; Wuana & Okieimen, 2011). When metals leach out from soil or sediments they become available in their ionic state and therefore become readily available to be carried across the membranes of organisms as well as in plant roots (Zeiner *et al.*, 2020; Chibuike *et al.*, 2014). Metals that have an affinity to organic matter in soil tend to become immobile and cannot move around and be readily accessible to some soil organisms (Chibuike *et al.*, 2014). When metals accumulate in the soil and sediments the bioavailability of these metals increases thus plants and micro-organisms may uptake/absorb more than they need (Wuana & Okieimen, 2011).

The bioavailability and mobility of metals in soil do not only affect plants and microbes but also contribute to environmental changes in the atmosphere thus affecting humans (Ashraf *et al.*, 2014). People can be affected by breathing in air contaminated by dust particles from soil and sediments containing trace/heavy metals and also by consuming crops and water grown from contaminated soil or sediments (Ashraf *et al.*, 2014; Barnett *et al.*, 2003; Chibuike *et al.*, 2014). The bioavailability of some metals in soil and sediments can indirectly add to essential trace elements such as Zn, Fe, and Mn, required by humans, animal, and plant biological systems (Olaniran *et al.*, 2013). The accumulation of non-essential trace/heavy metals in soil or sediment can be toxic to the environment, humans, animals, and plants (Rieuwerts *et al.*, 1998). Excessive exposure to trace/heavy metals such as cadmium can cause lung cancer (Aljahdali & Alhassan, 2020). Therefore soil quality and degree of contamination must be assessed to determine the quantity of trace/heavy metals in soil/sediments as well as to determine the mobility of these metals (Kim *et al.*, 2015). Because the duration of soil to metal interaction plays a role in the amount of metals present, it is more effective to assess the bioavailability of metals in soil rather than the overall quantity of metals (Takáč *et al.*, 2009). Therefore, one of the techniques that can be used to quantify the bioavailability of trace elements that can be toxic to plants and microorganisms in soil biota is the single extraction method (Zeiner *et al.*, 2020), this method is easy to conduct and requires fewer resources and equipment. However, the preparation is time-consuming because it uses various extraction solvents to evaluate the mobility of trace elements (Zeiner *et al.*, 2020).

This study aimed to determine the concentration of Al, As, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V using ICP-OES after pseudo-total digestion in sediments collected around the Sasolburg area, Free State Province, South Africa. Ultrasound-assisted single

extraction methods were used to investigate the mobility, bioavailability, and contamination levels of these metals. Environment assessments were used to help evaluate sediment quality and environmental impact in the study. To achieve this, various indices such as enrichment factor (E*f*), geo-accumulation index (Iego), contamination factor (C*f*), modified degree of contamination (m-C_d), bioavailability of heavy metals, and pollution load index (PLI) were utilized.

4.2 MATERIALS AND INSTRUMENTATION

4.2.1 Reagents and materials

Throughout the study, ultrapure water manufactured by Millipore Direct-Q 3UV-R water purification system (Merck) was utilized. Chemicals used in this study were of analytical reagent grade unless otherwise specified and used as supplied. Hydrochloric acid (37%) ultrapure nitric acid (70%), ethylenediaminetetraacetic acid (EDTA), acetic acid (99%), and calcium chloride were bought from Sigma-Aldrich (St. Loius MO, USA). For the preparation of calibration standards, 100 µg/mL stock solution (LGC, Teddington, UK) was used.

4.2.2 Instrumentation

Trace metals in sediment samples such as Al, As, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V were determined using an Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) (iCAP 6500 Duo, Thermo Scientific, UK). Bandelin sonorex digitech sonicator (Danbury, CT, USA) was used to carry out single extraction process. Isolation of the supernatant material from the sediment samples was done by using Eppendorf centrifuge 5702 (Eppendorf Ag, Hamburg Germany). pH was determined by using Ohaus, ST20 pH pen meter, and Conductivity was determined using Ohaus, Conductivity pen meter ST20C-B in a 1:5 ratio with distilled water. DigiBlock Digester ED36S, Labtech, Wilmington, MA was used to carry out pseudo-total digestion.

4.2.3 Study Area

The study was conducted in Meulsteenpan Lake (26.8692° S, 27.8806° E), Coalplex stream (26°49'41.4"S 27°53'45.6"E), and Natref stream (26°48'18.7"S 27°52'33.9"E) in Sasolburg as seen in Fig 4.1 which is situated in the north of the Free State Province (South Africa). Sasolburg is an industrial town that is sub-divided into Sasolburg, Vaalpark, and Zamdela

(Africa and State, 2020). Sasolburg is located on the east side of Taaibosspruit and adjacent to that stream are the industrial areas in the Vaal Triangle. Residential areas are south of the stream and farming practices are to the west (De Klerk, 2010).

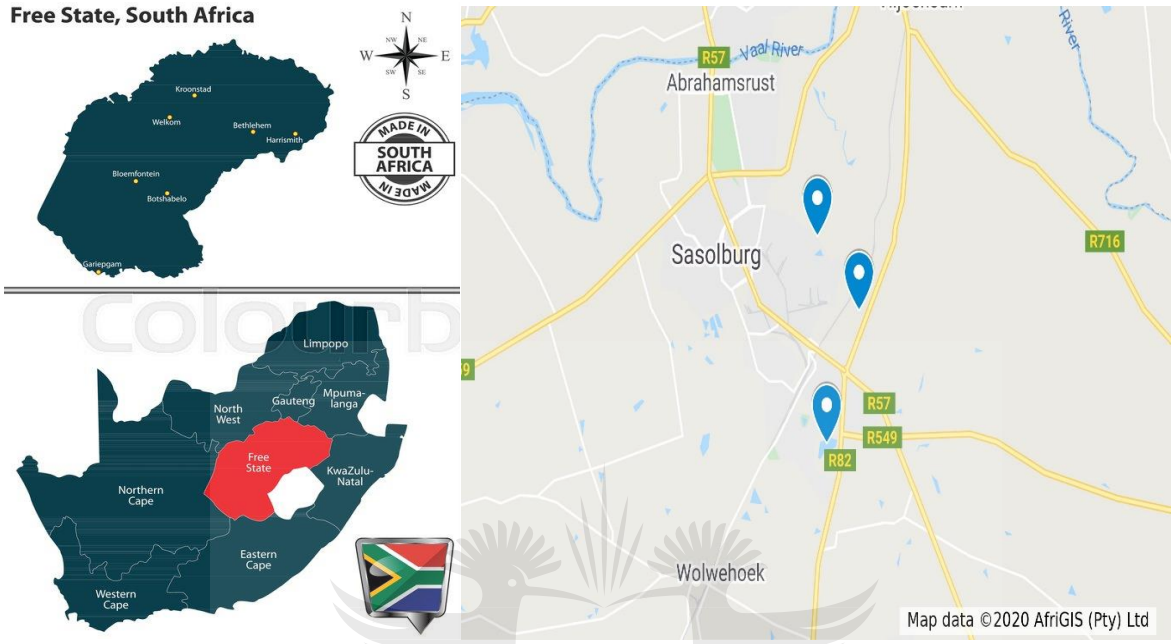

Figure 4.1: Map of South African provinces (Colouurbox, 2020) and sampling area (Google Maps)

4.2.4 Sampling collection

Sediment samples were collected from Meulsteenpan Lake, Coalplex stream, and Natref stream. A total of 27 samples were collected from all sampling sites. During sampling, a plastic scoop was used for the collection of sediment samples. Ziploc plastic bags were used for the storage of samples. Precautionary steps were implemented during the sampling process, this was required to avoid contamination of sediments samples and the use of metal-coated tools could have a considerable impact on sediments metal concentration. Samples were collected 20cm below ground level and transported in an icebox and stored in the refrigerator on arrival at the laboratory.

4.2.5 Sample preparation

Moisture content of sediment samples was initially determined, first, by weighing samples then allowing them to dry at room temperature after they have dried samples were reweighed again. To measure the overall moisture content, the mass after drying was subtracted by the

mass before drying, and the difference between the two masses was used to calculate the moisture content. The ratio between sediment and distilled water (1:5) was used to determine sediment pH. Dried samples were then crushed using a pestle and mortar and then sieved using a 425 mm mesh to obtain fine particles. Samples were then wrapped and sealed in Ziploc polyethylene bags until analysis.

4.2.6 Ultrasound-assisted single extraction technique

For the evaluation of bioavailable trace metals in sediments, several extraction solvents were used such as 0.11 mol L^{-1} CH_3COOH, 0.01 mol L^{-1} $CaCl_2$, 0.02 mol L^{-1} EDTA, and deionized water. An ultrasonic bath was used to support single extraction method. Samples were then centrifuged at 3000 rpm for 15 minutes. Sediment particles were then isolated from supernatant material using a polyvinylidene difluoride membrane filter (0.45 µm) (Sihlahla *et al.*, 2020).

Acetic acid extractant (0.1 mol L^{-1} CH_3COOH)

Precise measurements were weighed into 100 mL bottles.1.0 g of sediment samples were weighed and filled with a 40 mL aliquot of 0.11 mol L^{-1} CH_3COOH. The single extraction procedure was carried out in an ultrasonic bath for 1 hour.

Ethylenediaminetetraacetic acid extractant (EDTA, 0.02 mol L^{-1})

Approximately, 2 g of sediment samples were accurately weighed and transferred into 50 mL sample bottles followed by the addition of 20 mL 0.02 mol L^{-1} EDTA. The single extraction process was carried out in an ultrasonic bath for 1 hour.

Calcium chloride extractant (0.01 $CaCl_2$)

About 2 g of sediment samples were accurately weighed into 50 mL sample bottles and 20 mL of 0.01 mol L^{-1} $CaCl_2$ was added. The method was carried out in an ultrasonic bath for 1 hour.

Deionized water extractant

Sediment samples (about 2g) were accurately weighed and transferred into 50 mL sample bottles. A 20 mL aliquot of deionized water was added, and the extraction procedure was carried out in an ultrasonic bath for 1 hour.

4.2.7 Total digestion

The total digestion method was carried according to previous studies (Matong *et al.,* 2016; Sihlahla *et al.,* 2020). To describe the method briefly, 500 mg portions of dried sediment samples were accurately weighed and transferred into polypropylene digestive tubes followed by the addition of aqua regia (mixture of ultrapure HNO_3 and HCl at 1:3 ratio). The digestion tubes were sealed but not too tightly, then subjected into a one stage digestion program at 100 ˚C for 1 hour. Following the completion of the digestion process, samples were then taken out and allowed to cool to room temperature and then centrifuged at 3000 rpm to isolate supernatant from silicate residues (if there are any). The solution was then filtered using a polyvinyl difluoride membrane filter (0.45 µm) into a 100 mL volumetric flask and made to the mark with ultrapure water (Sihlahla *et al.*, 2020).

4.2.8 Assessment of heavy metal pollution

For the evaluation of sediment contamination and health risk, various environmental assessments were used in this study such as enrichment factor (E*f*), geo-accumulation index (Iego), contamination factor (C*f*), modified degree of contamination (m-C_d), bioavailability of heavy metals, and pollution load index (PLI).

4.3 RESULTS AND DISCUSSIONS

4.3.1 Physiochemical parameter of soil samples

Table 4.1 shows the summarized results of physiochemical parameters (pH, EC, and moisture content). Meulsteenpan Lake had a mean pH of 6.34 and 6.47 during the dry and wet seasons, respectively. The mean pH values for sediments collected from Coalplex and Natref Streams were more acidic in both dry (5.42 and 5.82) and wet (5.43 and 5.63) seasons (Table 4.1) as compared to Meulsteenpan Lake sediments. Table 4.1 shows that there are no significant seasonal changes in pH of the sediments in all three sampling sites. The pH values of the studies' sediments reflect slightly acidic conditions which suggest increased mobility of heavy/trace metals from sediment to the water system (Reicosky, 2018). Mean conductivity levels for Meulsteenpan Lake, Coalplex stream, and Natref stream during the dry season were found to be 0.42 mS/m, 0.4 mS/m, and 0.49 mS/m, respectively. While slightly higher EC values ranging from 0.47-0.53 mS/m during the wet season were recorded in all sites. The

moisture content ranged from 42-82% and 62-85% during the dry and wet seasons, respectively.

Table 4.1**:** Physiochemical parameters of soil samples

Parameters	**Meulsteenpan Lake**			**Coalplex Stream**			**Natref Stream**		
	Dry season								
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
pH	6.13	6.65	6.34	5.3	5.55	5.42	5.39	6.29	5.82
EC (mS/m)	0.26	0.53	0.42	0.18	0.74	0.4	0.18	1.02	0.49
Moisture (%)	42	82	57	65	74	69	78	81	80
	Wet season								
pH	6.01	6.75	6.47	5.11	5.98	5.43	5.41	5.82	5.63
EC (mS/m)	0.34	0.61	0.49	0.25	0.82	0.47	0.21	0.98	0.53
Moisture (%)	65	76	70	62	75	70	64	85	72

4.3.2 Total digestion

The total concentrations (minimum, maximum, and mean) of metals in sediments collected from Meulsteenpan Lake, Coalplex Stream and Natref Stream during wet and dry season are presented in Table 4.2 & 4.3. The trend of mean concentration of metals in Meulsteenpan Lake sediments was: Fe > Sr > Al > Ni > Cd > Cu > Mn > Se > Co > Pb > Tl > Mo > V > Cr > Sb > As during the dry season and during the wet season it was Fe > Sr > Al > Cd > Co > Pb > Ni > Mn > Se > Tl > Cu > Mo > Sb > Cr > V > As. The sediments collected in Coalplex stream had mean concentrations of metals in following this order: Fe > Mo > Sr > Mn > Pb > Al > V > Se > Cd > Co > Sb > Cu > Tl > Cr > As > Ni during the dry season and Fe > Al > Mo > Sr > Mn > Cd > Pb > V > Se > Co > Tl > Cu > Sb > Ni > Cr > As during the wet season. While for Natref stream the order was Fe > Sr > Mn > Al > Cd > Co > Cr > Se > Cu > Tl > V > As > Pb > Ni > Sb > Mo during the dry season and Fe > Al > Sr > Mn > Cd > Co > Se > Cr > Tl > Pb > Sb > Mo > As > Ni > Cu > V during wet season. The mean concentration of Fe, Mn, Cd, Co, Cu, Pb, and Ni exceeded the South African background level which suggest that their presence in the sediments might be due to anthropogenic activities. The average metal concentrations metals surpassing background level were in the order: Fe> Ni > Cd > Cu > Mn > Mn > Co > Pb for Meulsteenpan Lake, Fe > Mn > Pb > Cd > Co for Coalplex stream, and Fe > Mn > Cd > Co > Cu for Natref stream in the dry season,

for the wet season the order was: Fe > Cd > Co > Pb > Ni > Cu for Meulsteenpan Lake, Fe > Mn > Cd > Pb > Co for Coalplex stream, and Fe > Mn > Cd > Co for Natref stream. The Coalplex and Natref Streams are surrounded by industrial activities such as fossil fuel process and the levels of metal concentrations suggest that these environments are affected by these activities.

Table 4.2**:** Minimum, maximum, and mean of total concentrations (mg/kg) for metals in Meulsteenpan Lake, Coalplex Stream and Natref Stream sediments samples during the dry season

	Meulsteenpan Lake			**Coalplex Stream**			**Natref Stream**		
Elements	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
Al	1544	1907	1745	187	218	208	256	290	268
As	<DL	0.9	0.3	<DL	4.42	2.1	<DL	35.21	22.9
Cd	<DL	354	236	21.2	33.7	29.3	28.4	334.93	231
Co	40.5	41	40.8	14.2	28.1	23	3.72	312.75	208
Cr	9.42	12.4	10.5	0.94	10.8	5.2	39.7	87.9	60.3
Cu	176	188	182	2.44	21.6	9.76	24.8	75.36	45.5
Fe	6201	7555	6839	12162	13779	12846	11131	12018	11581
Mn	148	202	176	557	629.5	595	352	441	411
Mo	25.9	26.8	26.3	921	2132	1721	4.14	25	17.7
Ni	217	283	255	<DL	<DL	<DL	0.1	41	20.5
Pb	29.7	31.5	30.8	157	299	239	15	24.2	20.8
Sb	9.81	10.2	10	14.7	25.2	21.4	5.74	24.6	18.1
Se	58.4	59	58.7	54	68.5	63.54	35.3	67.2	56.1
Sr	3084	3523	3277	1396	1756	1558	5673	8546	6637
Tl	18.21	36.15	29.3	<DL	24.8	8.26	1.52	71.8	44.3
V	14.21	38.95	25.64	150	194	173	11.8	45.2	23.8

Table 4.3**:** Minimum, maximum, and mean of total concentrations (mg/kg) for metals in Meulsteenpan Lake, Coalplex Stream and Natref Stream sediments samples during the wet season

	Meulsteenpan Lake			**Coalplex Stream**			**Natref Stream**		
Elements	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
Al	1187	2433	1678	2378	4361	3337	2905	4858	3993
As	<DL	2.23	1.13	<DL	1.64	0.62	19.1	21.1	20.2
Cd	489	523	504	476	492	485	217	485.4	395
Co	428	512	483	36.7	46.7	42.8	152	195	179
Cr	17.5	23.1	20.7	4.12	21.5	13.1	40.6	67	55.6
Cu	25.8	39.1	31.5	23.6	29.9	27	3.87	31.5	14.9
Fe	4142	9352	6045	8594	13655	10984	13347	17157	15216
Mn	58.8	229	124	135	1274	601	408	719	518
Mo	28.2	30.3	29.4	962	1249	1094	25.5	28.37	23.7
Ni	173	219	195	13.9	28.1	22.8	2.22	32.58	19.2
Pb	208	644	412	142.2	219	185	19.2	36.86	29.4
Sb	27.4	28.8	27.9	27.5	28.8	28.3	25.6	28	26.7
Se	52.3	57.2	54.8	56.9	58	57.4	60.8	62.9	61.9
Sr	1894	2544	2305	808	1097	947	773	1356	1034
Tl	41.46	46.08	44.3	44	46	45	47.8	49.6	48.9
V	7.75	14.6	12.17	132	171	156	6.05	15.8	10.4

4.3.3 Assessment of trace/heavy metals mobility and bioavailability samples using single extraction method

The extractable fraction of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V in sediments were obtained using four different single extraction extractants (acetic acid, EDTA, $CaCl_2$, and deionised water). Figures 4.2 and 4.3 (Table 4A-1 – 4A - 2) revealed that the extractable fractions varied greatly among the different sampling sites, extractants and seasons. The extraction abilities of the metals by different extractants were found in the order EDTA > acetic acid > $CaCl_2$ > deionised water.

To determine the ability to extract metals from weakly bound soil/sediment biota, the extraction capacity of acetic acid was evaluated. Acetic acid extracted its highest concentrations in Ni, Fe, Mn, and Al in both dry and wet seasons Figure 4.2A and 4.3A. The acetic acid solution was able to extract other metals at low concentrations. Acetic acid gives some knowledge about the effect on trace/heavy metal mobility (Cappuyns & Science, 2012). Figures 4.2B and 4.3B revealed that $CaCl_2$ was able to extract most of the metals although at low concentrations. Calcium chloride is an unbuffered salt solution that is rapid, that can dissolve weakly adsorbed or exchangeable metals (Zhang *et al.*, 2020). For Fe and Mn, the extractable concentration using $CaCl_2$ was < 3 mg/kg for both metals, illustrating that the extractable fraction in the sediments was low. Fig. 4.2C2 and 4.3C2 revealed that the extractability of Fe and Mn were the highest when using the EDTA procedure as compared to other elements. EDTA can extract metals in non-silicate-bound soil, in which temporal and prolonged metal availability may be determined (Ure, 1996). The effective release of the majority of metals by EDTA is due to the fact that EDTA has a greater metal complexing potential. Deionised water was used as an extractant and was mostly able to extract Al and Fe as its highest elements during the study on both dry and wet seasons. Average metals extracted in the dry season had higher concentrations than those in the wet season, this may be due to the bioavailability in the soil biota. The use of deionised water in metal extractions offers advantages in determining the element capacity in soil solutions (Milićević *et al.*, 2017).

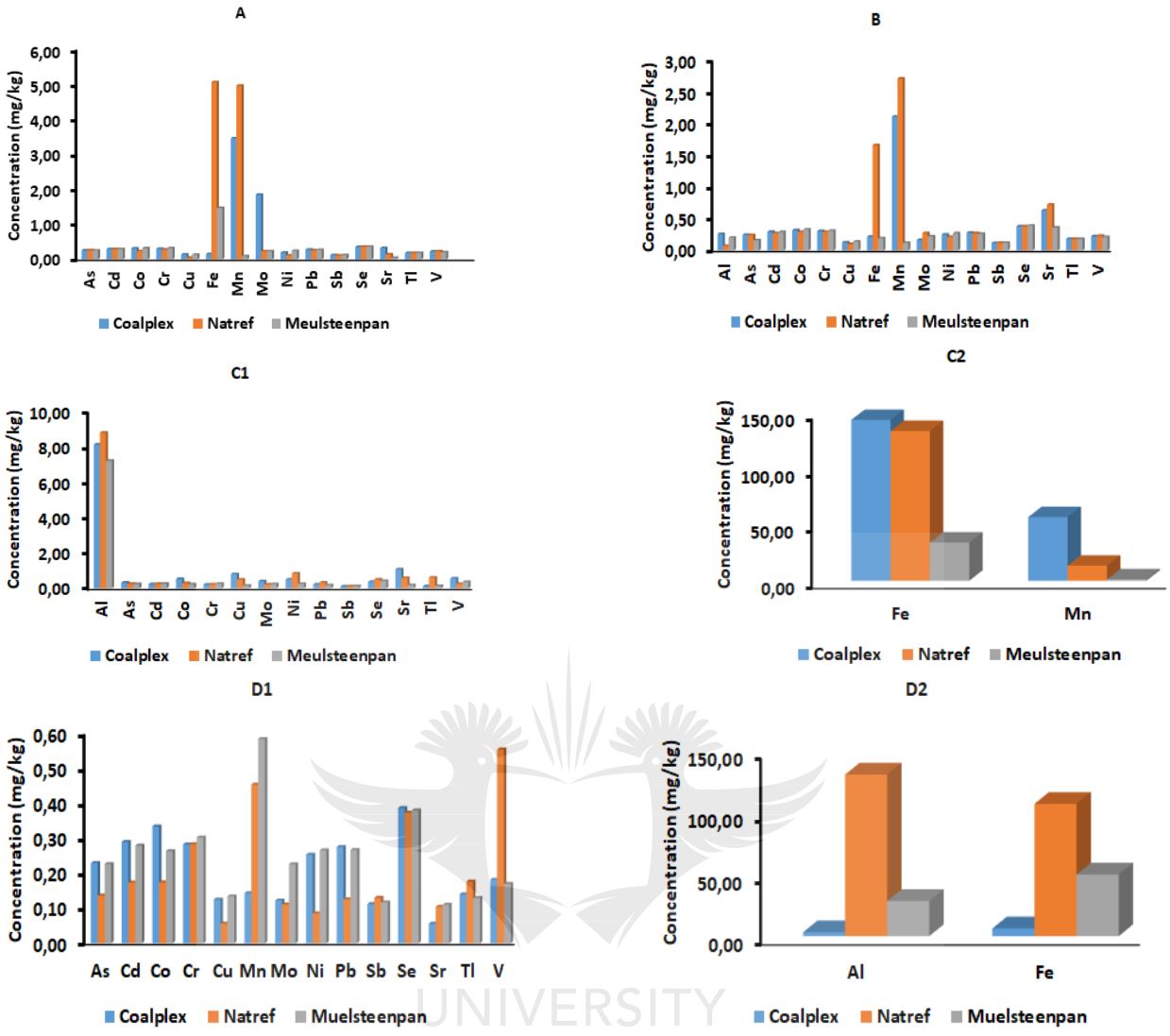

Figure 4.2: Concentration for all the metals in soil samples collected from Meulsteenpan Lake, Coalplex stream and Natref stream during the dry season: using A) Acetic Acid, B) Calcium Chloride, C1 & C2) EDTA, and D1 & D2) Deionized water

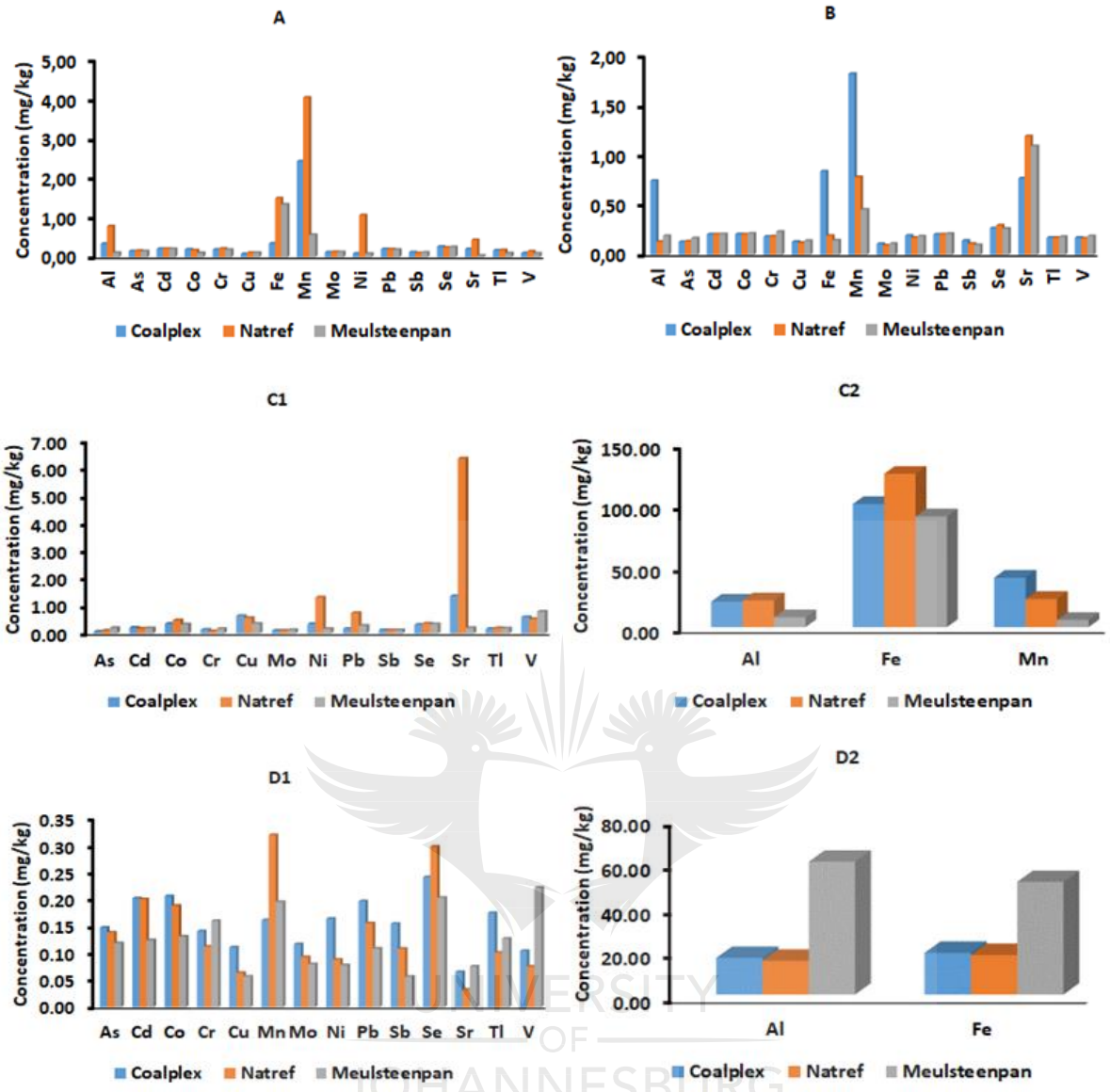

Figure 4.3: Concentration for all the metals in soil samples collected from Meulsteenpan Lake, Coalplex stream and Natref stream during the wet season: using A) Acetic Acid, B) Calcium Chloride, C1 & C2) EDTA, and D1 & D2) Deionized water

4.3.4 Bioavailability of heavy metals

4.3.4.1 Degree of bioavailability during the dry season

Table 4.4 present the results of expressing the degree of bioavailability of each metal ion extracted using acetic acid, $CaCl_2$, EDTA, and deionised water. In general, during the dry season, the degree of bioavailability values were less than one with exception of As in sediments from Coalplex stream and Meulsteenpan Lake. The degree of bioavailability values for Cr at Coalplex stream ranged 1.06-1.42%. Upon the evaluation of each extractant,

depending on the sampling site, the degree of bioavailability with EDTA was greater than or equal to 1% for the As, Cr, Cu, Mn, Ni, Pb, Fe, and V (Table 1, see bolded number). While for the degree of bioavailability values with acetic acid, As, Cr, Ni, and V were greater than 1%. The degree of bioavailability values with deionised water and CaCl2 were higher than 1 for As, Cr, and V (bolded values in Table 4). Therefore, during the dry season, the capabilities (In terms of the number of metals each reagent could extract) of the extractants to extract the available fractions of each metal were followed the order: EDTA> acetic acid > $CaCl_2$ = deionised water. These findings suggest that the bioavailability of trace metals in river sediments can be evaluated using EDTA extraction which was consistent with other studies (Teng *et al*, 2011).

4.3.4.1 Degree of bioavailability during the wet season.

As can be seen from Table 4.5, the degree of bioavailability with acetic acid, $CaCl_2$, EDTA, and deionised water ranged from 0-83%; 0-81%, 0-53%, and 0-76%, respectively. Therefore, the range of degree of bioavailability values obtained with different extractants followed the order of acetic acid > EDTA > deionised water > $CaCl_2$ (Table 4.5). Also, the bolded numbers in Table 5 suggested that EDTA and acetic acid could extract more metals from the sediments as compared to deionised water and $CaCl_2$. This might be due to the ability of EDTA and acetic acid to mimic the biochemical processes of aquatic plants. The bioavailable fraction of As was found to be higher in Coalplex stream and Meulsteenpan Lake followed by Cr. A similar trend was observed during the dry season. In comparison with the dry season, higher concentrations of various trace metals were bioavailable. This suggests that the wet conditions affected the interactions between the metals and organic matter of the sediments. Also, low to high concentrations of potentially toxic elements such as Tl, Pb and As were bioavailable (Table 4.4 & 4.5). Therefore, it can be concluded that when the conditions of the aquatic ecosystem change, the sediments in the study area have the potential to leach metals into water sources. Comparing the overall degree of bioavailability of all sites results followed the order: for dry season Coalplex stream > Meulsteenpan Lake > Natref Stream whereas for the wet season the order was: Meulsteenpan Lake > Coalplex stream > Natref

Table 4.4**:** Bioavailability of heavy metals in the dry season

Coalplex stream					**Natref stream**				**Meulsteenpan Lake**			
	AAC	EDTA	$CaCl_2$	DH_2O	AAC	EDTA	$CaCl_2$	DH_2O	AAC	EDTA	$CaCl_2$	DH_2O
Al	0.01	0.62	0.02	0.50	0.02	0.55	0.00	0.38	0.01	0.48	0.01	3.58
As	**23.5**	**10.2**	**20.8**	**23.9**	0.78	0.52	0.67	0.69	**12.9**	**17.7**	**14.6**	**10.5**
Cd	0.04	0.04	0.04	0.04	0.05	0.04	0.05	0.05	0.04	0.04	0.04	0.02
Co	0.45	0.82	0.48	0.48	0.10	0.27	0.11	0.11	0.02	0.07	0.04	0.03
Cr	**1.42**	**1.06**	**1.39**	**1.08**	0.38	0.14	0.33	0.20	0.90	0.78	**1.11**	0.78
Cu	0.28	**2.35**	0.48	0.41	0.73	**3.76**	0.81	0.43	0.34	**1.12**	0.45	0.18
Fe	0.00	0.91	0.01	0.17	0.01	0.82	0.00	0.12	0.02	**1.49**	0.00	0.84
Mn	0.40	**6.70**	0.30	0.03	0.78	**4.43**	0.15	0.06	0.45	**4.64**	0.37	0.16
Mo	0.01	0.01	0.01	0.01	0.53	0.42	0.40	0.39	0.42	0.42	0.38	0.27
Ni	0.38	**1.50**	0.84	0.72	**5.51**	**6.82**	0.89	0.46	0.04	0.08	0.10	0.04
Pb	0.11	0.09	0.11	0.11	0.66	**2.52**	0.70	0.53	0.04	0.07	0.05	0.03
Sb	0.42	0.43	0.50	0.55	0.37	0.43	0.41	0.41	0.41	0.42	0.35	0.20
Se	0.46	0.55	0.47	0.42	0.39	0.58	0.48	0.48	0.46	0.61	0.48	0.37
Sr	0.02	0.14	0.08	0.01	0.04	0.62	0.12	0.00	0.00	0.01	0.05	0.00
Tl	0.37	0.36	0.38	0.39	0.36	0.39	0.35	0.21	0.21	0.40	0.41	0.29
V	0.06	0.38	0.11	0.07	**1.38**	**5.00**	**1.59**	0.72	0.74	**6.49**	**1.54**	**1.82**

Table 4.5**:** Bioavailability of heavy metals in the wet season

Coalplex stream					**Natref stream**				**Meulsteenpan Lake**			
	AAC	EDTA	$CaCl_2$	DH_2O	AAC	EDTA	$CaCl_2$	DH_2O	AAC	EDTA	$CaCl_2$	DH_2O
Al	0.21	**3.92**	0.12	**1.51**	0.07	**3.29**	0.03	**48.7**	0.00	0.41	0.01	**1.63**
As	**12.2**	**15.4**	**11.6**	**10.9**	**1.14**	**1.12**	**1.05**	0.60	**83.3**	**81.1**	**53.3**	**75.5**
Cd	**1.00**	0.82	**1.00**	0.99	0.13	0.11	0.12	0.08	0.12	0.11	0.12	0.12
Co	**1.36**	**2.31**	**1.38**	**1.45**	0.11	0.14	0.14	0.08	0.78	0.57	0.81	0.65
Cr	**5.77**	**4.14**	**5.84**	**5.44**	0.46	0.36	0.48	0.47	**3.05**	**2.51**	**2.95**	**2.89**
Cu	**1.39**	**8.17**	**1.29**	**1.28**	0.11	**1.09**	0.22	0.12	0.07	0.08	0.08	0.07
Fe	0.00	**1.11**	0.00	0.05	0.04	**1.15**	0.01	0.92	0.02	0.50	0.00	0.73
Mn	0.58	**9.50**	0.35	0.02	1.21	**3.26**	0.66	0.11	0.05	0.71	0.07	0.33
Mo	0.11	0.02	0.01	0.01	1.30	**1.24**	**1.53**	0.63	0.87	0.89	0.84	0.86
Ni	0.00	0.00	0.00	0.00	0.54	**4.10**	**1.02**	0.42	0.09	0.10	0.11	0.10
Pb	0.12	0.09	0.12	0.12	1.25	**1.56**	**1.30**	0.60	0.88	0.57	0.84	0.87
Sb	0.55	0.51	0.54	0.52	0.61	0.63	0.66	0.72	**1.20**	**1.19**	**1.20**	**1.17**
Se	0.56	0.58	0.60	0.61	0.64	0.89	0.68	0.67	0.61	0.71	0.66	0.65
Sr	0.02	0.07	0.04	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.003
Tl	**2.20**	**1.43**	**2.19**	**1.69**	0.41	**1.37**	0.41	0.40	0.61	0.45	0.61	0.44
V	0.13	0.32	0.13	0.10	0.97	**1.03**	0.97	2.33	0.78	**1.41**	0.82	0.66

4.3.5 Environmental assessment

4.3.5.1 Contamination factor (Cf)

Table 4.6 represents the contamination factor for Cr, Ni, Pb, Cu, Co, Fe, and Mn in sediments collected during dry and wet seasons from the three study locations. As seen in Table 4.6 indicate that the C*f* for Cr falls in the low category (<1) in all the sampling sites. The C*f* for Ni fell into category 3 and 4 for Meulsteenpan Lake which represents a considerable and very high category for wet and dry seasons, while Coalplex and Natref stream fell into category 1 which represents low C*f* for the dry and wet season. During the dry season, the C*f* for Pb in Meulsteenpan Lake fell in the moderate category while at Coalplex stream fell in the very high category, and at Natref stream, the C*f* for Pb was low. During the wet season, the C*f* for Pb in Meulsteenpan Lake and Coalplex stream fell into category 4 which represents a very high C*f* while Natref stream had a moderate category.

The C*f* for Cu in Meulsteenpan Lake fell into category 4 (very high C*f*) and moderate category during the dry and wet season. While during dry season C*f* for Cu and Natref stream C*f* for Cu fell in low and moderate categories. In wet season C*f* for Cu in Coalplex and Natref stream had low C*f*. Cobalt in the dry season for Meulsteenpan Lake and Coalplex stream had moderate C*f*, while Natref stream had very high C*f*. In the wet season, Coalplex stream had moderate C*f* for Co while Meulsteenpan Lake and Natref stream fell into category 4 representing very high C*f*. Iron in the dry and wet seasons for Meulsteenpan Lake had considerable C*f*, while Coalplex and Natref stream had very high C*f* in both dry and wet seasons. Manganese in the dry season for Meulsteenpan Lake and Natref stream fell into category 2 which represented moderate C*f*, while Coalplex stream had considerable C*f*. In the wet season Mn for Meulsteenpan Lake had low C*f*, Coalplex stream had considerable C*f* and Natref stream fell into category 2 which represented moderate C*f*. Based on the results obtained on the soil samples, this suggests that the chosen sampling sites are impacted by anthropogenic activities.

Table 4.6**:** Contamination factor (C*f*) for sediment samples during the dry and wet season

	Dry			**Wet**		
Elements	Meulsteenpan Lake	Coalplex stream	Natref stream	Meulsteenpan Lake	Coalplex stream	Natref stream
Cr	0.15	0.07	0.84	0.29	0.18	0.77
Ni	6.72	0.00	0.54	5.13	0.60	0.51
Pb	1.42	11.0	0.96	19.0	8.54	1.35
Cu	6.16	0.33	1.54	1.07	0.92	0.51
Co	2.27	1.28	11.5	26.8	2.38	9.94
Fe	4.49	8.43	7.60	3.97	7.21	9.98
Mn	1.01	3.41	2.36	0.71	3.45	2.97

4.3.5.2 Modified degree of contamination (m-C_d)

Results in Table 7 indicate that in the dry season all sampling sites had a moderate degree of contamination, while in the wet season Meulsteenpan Lake had a high degree of contamination with Coalplex stream and Natref stream having a moderate degree of contamination. A positive about m-C_d is that it brings into consideration the mean value of all metals for a sampling site.

Table 4.7**:** Modified degree of contamination for all sample sites

Site	**Dry season**	**Wet season**
Meulsteenpan Lake	2.78	7.12
Coalplex Stream	3.07	2.91
Natref Stream	3.17	3.25

4.3.5.3 Pollution load index (PLI)

According to Table 4.8, results revealed that the PLI in Meulsteenpan Lake and Coalplex stream fell into category 2 indicating that they are fairly contaminated to uncontaminated, while Natref stream was in category 3 and was fairly contaminated. In the wet season, Coalplex stream and Natref stream fell into category 2 representing that they are fairly

contaminated to uncontaminated and Meulsteenpan Lake was in category 4 which indicated that it was fairly to extremely contaminated. This shows that sampling sites (especially Meulsteenpan Lake) are contaminated by anthropogenic activities.

Table 4.8**:** Pollution load index results

Site	Dry season	Wet season
Meulsteenpan Lake	1.90	3.02
Coalplex Stream	1.45	1.75
Natref Stream	2.02	1.87

4.3.5.4 Enrichment factor (Ef)

The E*f* was used to differentiate between the metals originating from anthropogenic (non-crustal) and geo-genic (crustal) sources (Shakeri *et al.*, 2014). The E*f* values for the sediment samples are represented in Fig 4. 4 and were used to evaluate trace/heavy metal enrichment at each sampling site in both dry and wet seasons. Based on the results obtained, Figure 4.4A had insufficiency to minimal enrichment. In Fig 4.4 B all elements experienced insufficiency to minimal enrichment except for Meulsteenpan Lake that had fair enrichment for Pb and considerable enrichment for Co. As mentioned in literature enrichment values < 2 are considered to be environmental inputs, while those > 2 are regarded as anthropogenic inputs (Shakeri *et al.*, 2014).

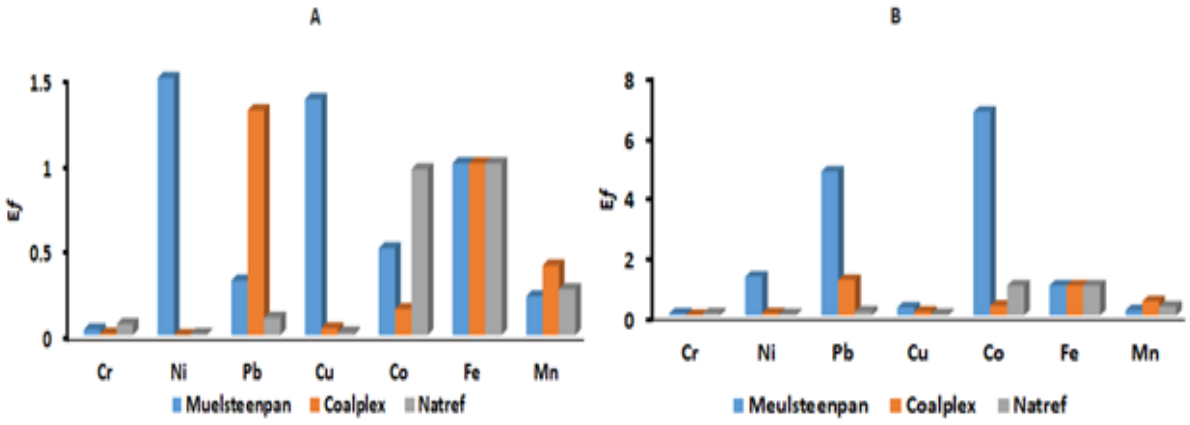

Figure 4.4: Enrichment factor (E*f*) for sediment samples collected from Meulsteenpan Lake, Coalplex stream and Natref stream during the A) dry season and B) Wet season.

4.3.5.5 Geo-accumulation index (Igeo)

Figure 4.5 represents Igeo results from all sample sites in both dry and wet seasons. In Fig 4.5 A & B, Cr had unavailability of contamination for all sample sites. Nickel for Meulsteenpan Lake had from fairly to strongly contaminated (Fig 4.5A and for Fig 4.5B), while for Coalplex stream and Natref stream Ni had unavailability of contamination. Meulsteenpan Lake and Natref stream experienced unavailability of contamination for Pb and Coalplex stream had from fairly to strongly contaminated (Fig 4.5A). In Fig 4.5B, Meulsteenpan Lake and Coalplex Stream was fairly to strongly contaminated by Pb while Natref stream had unavailability of Pb contamination. Meulsteenpan Lake was found to be fairly to strongly contaminated by Cu, while in Coalplex stream and Natref stream unavailable to fairly Cu contaminated (Fig 4.5A). Fig 4.5 B shows that there was unavailability of Cu contamination for all sampling sites. The Igeo values for Co suggested that the pollution in Meulsteenpan Lake ranged from unavailable to strongly contaminated (Fig 4.5). While for Coalplex stream was unavailable to fairly contaminated by Co and Natref stream was from fairly to strongly contaminated. In Fig 4.5, Coalplex stream and Natref stream were fairly to strongly contaminated by Fe while Meulsteenpan Lake was fairly contaminated by Fe. Fig 4.5 shows that the Igeo values for Mn in Meulsteenpan Lake, Coalplex stream, and Natref stream ranged from unavailability of contamination to fairly contaminated. Based on the Igeo results, it can be deduced that soil samples from all sampling sites were impacted by trace/heavy metal contamination because they experienced unavailability of contamination to extremely contaminated.

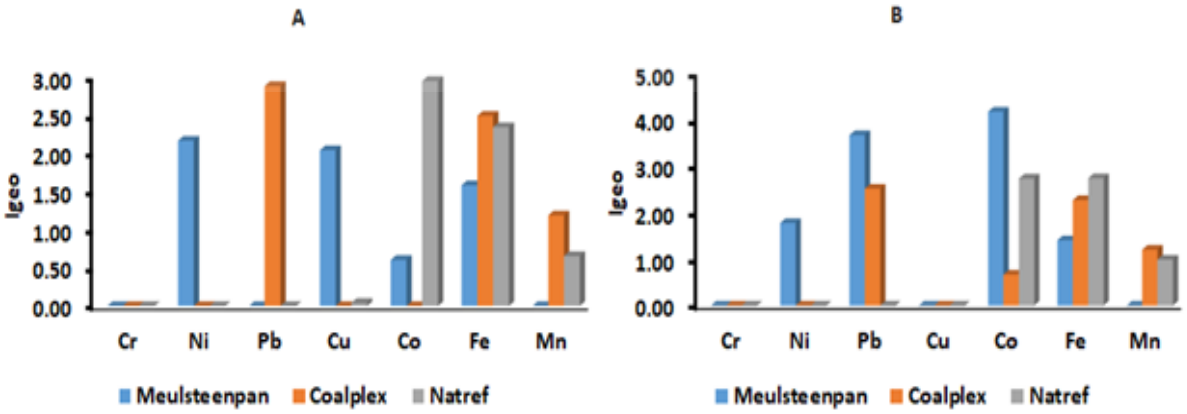

Figure 4.5: Geo-accumulation index (Igeo) for sediment samples collected from Meulsteenpan Lake, Coalplex stream and Natref stream during the A) dry season and B) Wet season.

4.4 CONCLUSION

The results obtained from single extraction methods and pseudo-total digestion on soil samples from Sasolburg suggested that the sediment samples contained major and trace elements originating from anthropogenic activities. The total concentration of Sr, Al, and Fe in Meulsteenpan Lake, Coalplex stream, and Natref stream were found to be relatively high during the dry and wet season. The single extraction procedure revealed that the highest concentrations of exchangeable fractions could be obtained when using EDTA, deionized water, and acetic acid. While calcium chloride extracted the lowest concentrations when compared to EDTA, deionized water, and acetic acid. Environmental risk assessments were evaluated using m-C_d, C*f*, E*f*, PLI, and Igeo, it was found based on the indices' categories from the selected study areas that surface water from all sites are highly contaminated by heavy metal pollution. Contamination factor (C*f*) showed that for both dry and wet seasons all sites were contaminated from low C*f* to very high C*f* for various metals. Modified degree of contamination indicated that in the dry season moderate degree of contamination was expected for all sample sites throughout and in the wet season Meulsteenpan Lake had a high degree of contamination while Coalplex stream and Natref stream had a moderate degree of contamination. Results obtained used PLI showed that is a source of contamination in sediment samples that may depreciate the environmental quality. Enrichment factor showed that in the dry season most metals were < 2 in all sampling sites while in the wet season Pb and Co had E*f* >2 indicating that there is anthropogenic contamination. Igeo was also used to help evaluate sediment contamination and with the observed results using Igeo it was found that unavailability of contamination to extremely contaminated was experienced when classified suggesting heavy metal contamination. Overall, the results of our studies have shown sediment degradation in an incremental trend that has the potential for adverse effects on the biogeochemical cycle, with potentially lethal implications for the survival of biodiversity.

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CHAPTER FIVE:
CONCLUSIONS AND RECOMMENDATION

5.1 GENERAL CONCLUSIONS

Due to their toxicity, the existence of trace/heavy metals in the environment is of significant concern even though they exist in nature at low concentrations. The increase of trace/heavy metals in the environment is due to anthropogenic activities such as mining, agricultural activities, and industrial activities. This research was therefore carried out to determine the pollution and bioavailability of trace/heavy metals in soil/sediments and surface water obtained from South Africa, Free State, Sasolburg due to several anthropogenic activities as mentioned above. To determine the possible mobility, bioavailability, and transfer of trace/heavy metals from soil/sediments to its surroundings (surface water), ultrasonic single extraction procedures and pseudo-total digestions were utilized for soil/sediment samples and the metals were quantified using the ICP-OES. Although conclusions were reached for each chapter, a general conclusion was made in relation to the overall study as well as to the objectives outlined in the following paragraph. In addition, it briefly addresses recommendations and suggestions for future work for this dissertation.

The first objective of the study was the quantification of heavy metal concentration levels (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Tl, and V) measured from water samples collected in Sasolburg, Free State (South Africa) and to assess the environmental impact caused by these metals by using various pollution indices. The first objective was fully met, which is found in chapter three of the study. Results obtained from this study revealed that the impact of contamination in surface waters. It was noticed that physiochemical parameters for each season in all sampling locations experienced seasonal variations in some locations for the dry season pH, EC, and TDs were lower than the wet season and vice versa. This may be due to the introduction of high waste from industries since the amount of rainwater is greater in the wet season in Sasolburg compared to the dry season. Biogeochemical processes may also influence physiochemical parameters. Factors such as Algae influence pH, EC, and TDS of water content. During the wet season, there has been an increase in plants growing within the stream and lake. The plant roots and biofilm-associated with them can profoundly influence the chemistry of the

surface water. The chemicals produced by plants can influence the uptake of metals in surface water, and thus influence physiochemical parameters.

Surface water from the Coalplex stream had a high concentration for Fe, Mn, and Mo, while from Meulsteenpan Lake Al, Fe, and Mn were the highest, and in the Natref stream, the highest concentrations were from Fe and Mn. In Coalplex stream wet season, Fe surpassed the water quality guideline values set by DWAF and EPA domestic use, Mn exceeded water quality guideline values set by DWAF and EPA for domestic use and DWAF agricultural use, while Mo had high concentration dry and wet season and exceeding water quality guideline values set by DWAF agricultural use. In Meulsteenpan Lake wet season, Al and Fe exceeded water quality guidelines set by DWAF and EPA for domestic use, while Mn exceeded water quality guideline values for DWAF and EPA for both domestic and agricultural use. In the Natref stream wet season, high Fe concentrations were analyzed and found to be higher than the water quality guideline value set by DWAF domestic use, while Mn had high concentrations and exceeded water quality guideline values set by DWAF and EPA domestic use and DWAF agricultural use. Based on the study done, the highest concentrations were witnessed in the wet season suggesting that the most contamination was highly active in the wet season. Various environmental risk assessments (HEI, m-HPI, NeI, C*f*, and m-C_d) were used to evaluate heavy metal contamination. For example, the C*f* values for As showed moderate contamination (moderate ($1 \leq Cf \leq 3$) in Natref and Coalplex. Suggesting that the source of As in surface water comes from anthropogenic activities. The C*f* values for the rest of the investigated metals displayed low contamination (C*f* <1) in all sampling sites except Se in Meulsteenpan Lake. Modified degree of contamination for this study exhibited moderate, low, and nil to a very low degree of contamination. This also shows that most contamination was experienced in the wet season These environmental assessments suggested the surface water was contaminated with the presence of heavy metals.

The second objective was the assessment of trace/heavy metal concentrations in sediment samples collected from Sasolburg, Free State (South Africa) by using ultrasound assisted single extraction and pseudo-total digestion techniques aided by the ICP-OES. The use of different pollution assessments to help evaluate the mobility and bioavailability of these trace metals. Sediment physiochemical parameters were measured such as pH and EC. Sediment pH is an important parameter affecting the solubility and mobility of metal fractions. The pH values found

from sediment samples revealed that they were slightly acidic suggesting that there is a presence of trace/heavy metal contamination. pH has the potential to modify metal solubility/availability in several ways including dissolution. The decrease in sediment metal content during the wet season may be influenced by the low pH levels recorded. EC was higher in the wet season this is possibly due to the leaching of soluble salts by runoff water from the water bodies. Extractants like deionized water, EDTA, acetic water, and calcium chloride were used to assist with single extraction technique. The extraction abilities of the metals by different extractants were found in the order EDTA > acetic acid > $CaCl_2$ > deionised water. Findings from this study indicate that trace/heavy metals from sediments are mobile and bioavailable based on EDTA, $CaCl_2$, deionized water, and acetic acid. The degree of bioavailability with acetic acid, $CaCl_2$, EDTA, and deionised water ranged from 0-83%; 0-81%, 0-53%, and 0-76%, respectively with the range of degree of bioavailability values obtained with different extractants following the order of acetic acid > EDTA > deionised water > $CaCl_2$. Environmental risk assessments (C*f*, m-C_d, Igeo, E*f*, PLI, and Bioavailability) were also used to help assess trace/heavy metal contamination and bioavailability in the environment to reveal the impact and risks associated with trace/heavy metal contamination. Contamination factors for the selected metals (Cr, Ni, Pb, Cu, Co, Fe, and Mn) in sediment samples varied from low C*f* to very high C*f*, which suggests that selected study areas around Sasolburg are affected by anthropogenic activities. The enrichment factor **(**E*f*) was used to differentiate between trace metals originating from anthropogenic (non-crustal) and geo-genic (crustal) sources. Meulsteenpan Lake had fair enrichment for Pb and considerable enrichment for Co, which suggests high amounts of trace metals from anthropogenic sources. It is evident that water and sediment from Meulsteenpan Lake, Coalplex Stream, and Natref stream were polluted with trace metals based on the above points, and that the use of these surface waters is harmful to human health, environment for recreational purposes, laundry, and fishing. Significant and important steps are therefore needed to monitor, treat and maintain the water and sediment quality.

5.2 RECOMMENDATIONS

A regular review of the use of complex matrices (soil/sediments and surface water) for controlling the level of toxicity of metals and the risk assessment of urban and industrial areas

has proven to be enough for the methods used in this research. However, to know the reasons leading to metal translocation, a more rigorous and in-depth study is needed. A correlation with the medical data given by local clinics and hospitals will also help to explain the effect on human health of these trace/heavy metals.

APPENDICES

Chapter 3

Table 3 A-1: Contamination factor set by DWAF (domestic use)

Dry season				Wet season		
	Coalplex	**Natref**	**Meulsteenpan**	**Coalplex**	**Natref**	**Meulsteenpan**
Al	0.18	0.27	0.18	0.63	0.97	2.42
As	1.02	1.06	0.06	0.43	0.21	0.36
Cd	0.011	0.15	0.21	0.04	0.015	0.07
Co	N. A	N.A	N.A	N.A	N.A	N.A
Cr	0.03	0.04	0.04	0.006	0.04	0.08
Cu	0.005	0.006	0.008	0.002	0.008	0.004
Fe	0.52	0.05	N.A	2.79	1.15	20.7
Mn	0.70	0.81	0.22	5.43	1.21	11.4
Mo	N.A	N.A	N.A	N.A	N.A	N.A
Ni	N.A	N.A	N.A	N.A	N.A	N.A
Pb	0.09	0.32	0.27	0.38	0.22	0.22
Sb	N.A	N.A	N.A	N.A	N.A	N.A
Se	0.37	0.41	0.78	0.31	0.32	0.78
Sr	N.A	N.A	N.A	N.A	N.A	N.A
Ti	N.A	N.A	N.A	N.A	N.A	N.A
V	0.15	0.15	0.14	0.09	0.13	0.22

N.A = Not Available

Table 3 A-2: Contamination factor set by EPA (domestic use)

Dry season				Wet season		
	Coalplex	**Natref**	**Meulsteenpan**	**Coalplex**	**Natref**	**Meulsteenpan**
Al	0.134	0.21	0.14	0.47	0.72	0.65
As	1.02	1.06	0.062	0.43	0.22	1.97
Cd	0.01	0.15	0.21	0.04	0.02	2.88
Co	N.A	N.A	N.A	N.A	N.A	N.A
Cr	0.03	0.04	0.04	0.006	0.04	0.161
Cu	0.002	0.003	0.004	0.001	0.004	0.32
Fe	0.26	0.02	N.A	1.39	0.57	2.42
Mn	0.70	0.81	0.22	5.43	1.21	4.50
Mo	N.A	N.A	N.A	N.A	N.A	N.A
Ni	N.A	0.03	0.034	N.A	N.A	N.A
Pb	0.09	0.32	0.27	0.38	0.22	1.77
Sb	0.45	0.17	0.21	0.70	0.30	2.30
Se	0.75	0.83	1.55	0.63	0.65	1.00
Sr	N.A	N.A	N.A	N.A	N.A	N.A
Ti	N.A	N.A	N.A	N.A	N.A	N.A
V	N.A	N.A	N.A	N.A	N.A	N.A

N.A = Not Available

Table 3 A-3: Contamination factor set by WHO (domestic use)

Dry season				**Wet season**		
	Coalplex	**Natref**	**Meulsteenpan**	**Coalplex**	**Natref**	**Meulsteenpan**
Al	N.A	N.A	N.A	N.A	N.A	N.A
As	1.02	1.06	0.06	0.43	0.22	1.97
Cd	0.02	0.26	0.34	0.07	0.02	2.88
Co	N.A	N.A	N.A	N.A	N.A	N.A
Cr	0.03	0.04	0.04	0.006	0.04	0.16
Cu	0.002	0.003	0.004	0.001	0.004	0.32
Fe	N.A	N.A	N.A	N.A	N.A	N.A
Mn	N.A	N.A	N.A	N.A	N.A	N.A
Mo	N.A	N.A	N.A	N.A	N.A	N.A
Ni	N.A	0.01	0.01	N.A	N.A	N.A
Pb	0.09	0.32	0.27	0.38	0.22	1.77
Sb	0.11	0.04	0.05	0.17	0.08	2.30
Se	0.18	0.21	0.39	0.16	0.16	1.00
Sr	N.A	N.A	N.A	N.A	N.A	N.A
Ti	N.A	N.A	N.A	N.A	N.A	N.A
V	N.A	N.A	N.A	N.A	N.A	N.A

N.A = Not Available.

Table 3 A-4: Contamination factor set by DWAF (agricultural use)

Dry season				**Wet season**		
	Coalplex	**Natref**	**Meulsteenpan**	**Coalplex**	**Natref**	**Meulsteenpan**
Al	0.01	0.01	0.01	0.02	0.03	0.07
As	0.10	0.11	0.01	0.04	0.02	0.04
Cd	0.01	0.08	0.10	0.02	0.01	0.04
Co	0.04	0.18	0.18	0.22	0.13	0.05
Cr	0.01	0.02	0.02	N.A	0.02	0.04
Cu	0.02	0.03	0.04	0.01	0.04	0.02
Fe	0.01	N.A	N.A	0.06	0.02	0.42
Mn	0.70	0.81	0.22	5.43	1.21	11.36
Mo	34.12	0.33	0.71	20.80	0.34	0.76
Ni	N.A	N.A	N.A	N.A	N.A	N.A
Pb	N.A	0.02	0.01	0.02	0.01	0.01
Sb	N.A	N.A	N.A	N.A	N.A	N.A
Se	0.37	0.41	0.78	0.31	0.32	0.78
Sr	N.A	N.A	N.A	N.A	N.A	N.A
Ti	N.A	N.A	N.A	N.A	N.A	N.A
V	0.15	0.15	0.14	0.09	0.13	0.22

N.A = Not Available

Chapter 4

Table 4 A-1: Single extraction results (dry season) in mg/kg

	Acetic acid			**$CaCl_2$**			**EDTA**			**Deionised water**		
	Coalplex	Natref	Meulsteenpan	Coalplex	Natref	Meulsteenpan	Coalplex	Natref	Meulsteenpan	Coalplex	Natref	Meulsteenpan
Al	0.45	0.18	0.06	0.26	0.07	0.2	8.14	8.81	7.22	3.15	130.54	28.41
As	0.26	0.26	0.25	0.24	0.24	0.16	0.32	0.26	0.24	0.23	0.14	0.23
Cd	0.29	0.29	0.29	0.29	0.27	0.29	0.24	0.25	0.26	0.29	0.17	0.28
Co	0.31	0.23	0.32	0.32	0.29	0.33	0.53	0.29	0.23	0.33	0.17	0.26
Cr	0.30	0.28	0.32	0.30	0.29	0.31	0.22	0.22	0.26	0.28	0.28	0.30
Cu	0.14	0.05	0.13	0.13	0.1	0.14	0.80	0.50	0.15	0.12	0.06	0.13
Fe	0.15	5.07	1.47	0.21	1.66	0.19	142.80	132.99	33.94	6.26	106.81	49.79
Mn	3.46	4.97	0.09	2.11	2.71	0.12	56.54	13.38	1.25	0.14	0.45	0.59
Mo	1.85	0.23	0.23	0.16	0.27	0.22	0.40	0.22	0.23	0.12	0.11	0.23
Ni	0.19	0.11	0.24	0.25	0.21	0.27	0.50	0.84	0.25	0.25	0.09	0.27
Pb	0.28	0.26	0.27	0.28	0.27	0.26	0.22	0.33	0.18	0.28	0.13	0.27
Sb	0.12	0.11	0.12	0.12	0.12	0.12	0.11	0.11	0.12	0.11	0.13	0.12
Se	0.35	0.36	0.36	0.38	0.38	0.39	0.37	0.50	0.41	0.39	0.37	0.38
Sr	0.32	0.14	0.04	0.63	0.72	0.36	1.07	0.58	0.18	0.06	0.10	0.11
Tl	0.18	0.18	0.18	0.18	0.18	0.18	0.12	0.61	0.13	0.14	0.18	0.13
V	0.22	0.23	0.2	0.22	0.23	0.21	0.56	0.25	0.36	0.18	0.56	0.17

Table 4 A-2: Single extraction results (wet season) in mg/kg

	Acetic acid			**$CaCl_2$**			**EDTA**			**Deionised water**		
	Coalplex	Natref	Meulsteenpan	Coalplex	Natref	Meulsteenpan	Coalplex	Natref	Meulsteenpan	Coalplex	Natref	Meulsteenpan
Al	0.34	0.78	0.11	0.74	0.13	0.19	20.80	21.83	8.02	16.54	15.29	60.01
As	0.15	0.16	0.15	0.13	0.14	0.17	0.06	0.105	0.20	0.15	0.14	0.12
Cd	0.21	0.21	0.21	0.20	0.21	0.21	0.21	0.175	0.19	0.20	0.20	0.12
Co	0.19	0.17	0.10	0.21	0.21	0.21	0.35	0.475	0.33	0.21	0.19	0.13
Cr	0.19	0.21	0.19	0.18	0.19	0.23	0.14	0.08	0.16	0.14	0.11	0.16
Cu	0.08	0.11	0.11	0.13	0.12	0.14	0.64	0.56	0.35	0.11	0.06	0.06
Fe	0.34	1.49	1.33	0.84	0.19	0.14	100.09	124.685	90.04	18.81	17.80	50.85
Mn	2.43	4.04	0.56	1.82	0.78	0.45	40.25	22.94	5.76	0.16	0.32	0.20
Mo	0.12	0.13	0.12	0.11	0.10	0.11	0.10	0.1	0.12	0.12	0.09	0.08
Ni	0.09	1.06	0.08	0.19	0.17	0.19	0.34	1.31	0.16	0.16	0.09	0.08
Pb	0.20	0.20	0.18	0.20	0.21	0.21	0.17	0.74	0.28	0.20	0.16	0.11
Sb	0.12	0.10	0.11	0.14	0.11	0.10	0.12	0.115	0.12	0.15	0.11	0.06
Se	0.26	0.24	0.25	0.27	0.30	0.26	0.31	0.36	0.33	0.24	0.30	0.20
Sr	0.20	0.43	0.04	0.77	1.19	1.09	1.35	6.36	0.20	0.07	0.03	0.08
Tl	0.17	0.17	0.09	0.17	0.17	0.18	0.16	0.19	0.18	0.17	0.10	0.13
V	0.10	0.14	0.09	0.17	0.17	0.19	0.59	0.52	0.79	0.10	0.08	0.22

Table 4 A-3: Enrichment factor results

Dry season				Wet season		
	Meulsteenpan	**Coalplex**	**Natref**	**Meulsteenpan**	**Coalplex**	**Natref**
Cr	0.03	0.01	0.06	0.07	0.03	0.08
Ni	1.50	0.00	0.01	1.29	0.08	0.05
Pb	0.32	1.31	0.10	4.78	1.18	0.14
Cu	1.37	0.04	0.01	0.27	0.13	0.05
Co	0.51	0.15	0.97	6.77	0.33	1.00
Fe	1.00	1.00	1.00	1.00	1.00	1.00
Mn	0.23	0.41	0.27	0.18	0.48	0.30

N.A = Not available

Table 4 A-4: Geo-accumulation results

Dry season				Wet season		
	Meulsteenpan	**Coalplex**	**Natref**	**Meulsteenpan**	**Coalplex**	**Natref**
Cr	N.A	N.A	N.A	N.A	N.A	N.A
Ni	2.16	N.A	N.A	1.77	N.A	N.A
Pb	N.A	2.88	N.A	3.66	2.51	N.A
Cu	2.04	N.A	0.04	N.A	N.A	N.A
Co	0.60	N.A	2.94	4.16	0.66	2.73
Fe	1.58	2.49	2.34	1.40	2.26	2.73
Mn	N.A	1.19	0.65	N.A	1.20	0.99

N.A = Not available

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